1. **Dipeptidyl peptidase 4 (EC: 3.4.14.5)**

Reaction:

Release of an N-terminal dipeptide, Xaa-Yaa-|-Zaa-, from a polypeptide, preferentially when Yaa is Pro, provided Zaa is neither Pro nor hydroxyproline.

Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to assay buffer (50 mM sodium phosphate, pH = 7.4), and the reaction was started by the addition of substrate (H-A-pNA, Cfinal = 250 μM). After incubation for 30 min at 37°C, the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (H-A-pNA).

Reference:

L. Dai et al., Analysis of the Structure and Activity of Dipeptidyl Peptidase IV (DPP-IV) Inhibitory Oligopeptides from Sorghum Kafirin. J Agric Food Chem 70, 2010-2017 (2022).

1. **Monoamine oxidase (EC: 1.4.3.4)**

Reaction:

a secondary aliphatic amine + H2O + O2 = a primary amine + an aldehyde + H2O2



Reaction system: Based on the protocol of the detection kit (Biotides Cat# TE0253). Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to assay buffer and reaction with benzylamine (Cfinal = 20 mM) to generate benzaldehyde, and then added 2,4-dinitrophenylhydrazine chromogenic reagent to detect benzaldehydeAfter incubation for 30 min at 37°C, the absorbance was measured at 470 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (benzylamine).

Reference:

G. Huang *et al.*, A spectrophotometric assay for monoamine oxidase activity with 2, 4-dinitrophenylhydrazine as a derivatized reagent. *Analytical Biochemistry* **512**, 18-25 (2016).

1. **Biliverdin reductase (EC:** **1.3.1.24)**

Reaction:

bilirubin IXalpha + NAD+ = biliverdin IXalpha + H+ + NADH



Reaction system: Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to assay buffer containing NADH (Cfinal = 200 μM), and the reaction was started by the addition of substrate (biliverdin, Cfinal = 10 μM). After incubation for 30 min at 37°C, the absorbance was measured at 453 nm (bilirubin). For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (bilirubin).

Reference:

R. van Dijk et al., Biliverdin Reductase inhibitors did not improve severe unconjugated hyperbilirubinemia in vivo. Sci Rep 7, 1646 (2017).

1. **Tyrosine transaminase (****EC: 2.6.1.5)**

Reaction:

2-oxoglutarate + L-tyrosine = 3-(4-hydroxyphenyl) pyruvate + L-glutamate



Reaction system: Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing tyrosine (Cfinal = 3 mM), 2-oxoglutarate (Cfinal = 3 mM), 50 μM PLP. After incubation for 30 min at 37°C, stopped by 0.2 ml 10 M NaOH. The absorbance at 331 nm (p-hydroxyphenylpyruvic acid converted to p-hydroxybenzaldehyde in strong alkali) was measured after 30 min at room temperature. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (2-oxoglutarate and L-tyrosine).

Reference:

J. Dundjerski, B. Butorović, J. Kipić, D. Trajković, G. Matić, Cadmium affects the activity of rat liver tyrosine aminotransferase and its induction by dexamethasone. Arch Toxicol 70, 390-395 (1996).

1. **Carboxylesterase (EC:** **3.1.1.1)**

Reaction:

a carboxylic ester + H2O = a carboxylate + an alcohol



Reaction system: Based on the protocol of the detection kit (Solarbio Cat# BC0840). Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to reaction buffer and reaction with naphthyl acetate to generate naphthalene ester, and then added fast blue salt chromogenic agent. After incubation for 30 min at 37°C, the absorbance was measured at 450 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (naphthyl acetate).

Reference:

N. Thangthaeng, N. Sumien, M. J. Forster, R. A. Shah, L. J. Yan, Nongradient blue native gel analysis of serum proteins and in-gel detection of serum esterase activities. J Chromatogr B Analyt Technol Biomed Life Sci 879, 386-394 (2011).

1. **Membrane alanyl aminopeptidase (EC: 3.4.11.2)**

Reaction:

Release of an N-terminal amino acid, Xaa-|-Yaa- from a peptide, amide or arylamide. Xaa is preferably Ala, but may be most amino acids including Pro (slow action). When a terminal hydrophobic residue is followed by a prolyl residue, the two may be released as an intact Xaa-Pro dipeptide.

Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and the reaction was started by the addition of substrate (L-leu-pNA; Cfinal = 200 μM). After incubation for 30 min at 37°C, the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (L-leu-pNA).

Reference:

M. Niu, F. Wang, F. Li, Y. Dong, Y. Gu, Establishment of a screening protocol for identification of aminopeptidase N inhibitors. J Taiwan Inst Chem Eng 49, 19-26 (2015).

1. **Carbonyl reductase (NADPH) (EC: 1.1.1.184)**

Reaction:

a ketone + H+ + NADPH = a secondary alcohol + NADP+



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to 0.1M NaH2PO4 buffer, and the reaction was started by the addition of 2, 3-hexanedione (Cfinal = 500 μM), and NADPH (Cfinal = 200 μM). After incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (2, 3-hexanedione).

Reference:

J. M. Seliger, H. J. Martin, E. Maser, J. Hintzpeter, Potent inhibition of human carbonyl reductase 1 (CBR1) by the prenylated chalconoid xanthohumol and its related prenylflavonoids isoxanthohumol and 8-prenylnaringenin. Chem Biol Interact 305, 156-162 (2019).

1. **Arylesterase (EC: 3.1.1.2)**

Reaction:

a phenyl acetate + H2O = a phenol + acetate + H+



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing phenylacetate (Cfinal = 1.0 mM). After incubation for 30 min at 37°C, the absorbance was measured at 270 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (phenylacetate).

Reference:

A. Mahrooz, M. R. Rashidi, M. Nouri, Naringenin is an inhibitor of human serum paraoxonase (PON1): an in vitro study. J Clin Lab Anal 25, 395-401 (2011).

1. **Phosphoglycerate dehydrogenase (EC: 1.1.1.95)**

Reaction:

(2R)-3-phosphoglycerate + NAD+ = 3-phosphooxypyruvate + H+ + NADH



Reaction system: Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing (2R)-3-phosphoglycerate (Cfinal = 240 μM), NAD+ (Cfinal = 200 μM). After incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate ((2R)-3-phosphoglycerate).

Reference:

E. Mullarky et al., Identification of a small molecule inhibitor of 3-phosphoglycerate dehydrogenase to target serine biosynthesis in cancers. Proc Natl Acad Sci U S A 113, 1778-1783 (2016).

1. **Choline O-acetyltransferase (EC: 2.3.1.6)**

Reaction:

acetyl-CoA + choline = acetylcholine + CoA



Reaction system: Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to Tris buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1.0 mM EDTA, 0.05% (v / v) Triton X-100) containing choline (Cfinal = 150 μM), acetyl-CoA (Cfinal = 15 μM), diethylamino-3-(4-maleimidophenyl)-4-methylcoumarin (CPM; Cfinal = 15 μM). After incubation for 20 min at 37°C, the fluorescence intensity was measured at λex=390 nm, λem=479 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (choline and acetyl-CoA).

Reference:

R. Kumar, A. Kumar, B. Långström, T. Darreh-Shori, Discovery of novel choline acetyltransferase inhibitors using structure-based virtual screening. Sci Rep 7, 16287 (2017).

1. **Nicotinamide-nucleotide adenylyltransferase (EC: 2.7.7.1)**

Reaction:

ATP + beta-nicotinamide D-ribonucleotide + H+ = diphosphate + NAD+



Reaction system: Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer (pH = 7.4) containing ATP (Cfinal = 2 mM), Alcohol Dehydrogenase (Cfinal = 6 U/ml), alcohol (Cfinal = 60 mM), beta-nicotinamide D-ribonucleotide (Cfinal = 2 mM). After incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (nicotinamide mononucleotide).

Reference:

L. Sorci et al., Nicotinamide mononucleotide synthetase is the key enzyme for an alternative route of NAD biosynthesis in Francisella tularensis. Proc Natl Acad Sci U S A 106, 3083-3088 (2009).

1. **UDP-glucose 6-dehydrogenase (EC: 1.1.1.22)**

Reaction:

H2O + 2 NAD+ + UDP-alpha-D-glucose = 3 H+ + 2 NADH + UDP-alpha-D-glucuronate



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing uridine 5'-diphosphoglucose disodium salt (Cfinal = 100 μM), NAD+ (Cfinal = 200 μM). After incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (uridine 5'-diphosphoglucose disodium salt).

Reference:

S. Scoglio et al., Inhibitory effects of Aphanizomenon flos-aquae constituents on human UDP-glucose dehydrogenase activity. J Enzyme Inhib Med Chem 31, 1492-1497 (2016).

1. **Cystathionine beta-synthase (EC: 4.2.1.22)**

Reaction:

L-homocysteine + L-serine = H2O + L,L-cystathionine



Reaction system: Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing PLP (Cfinal = 100 μM), L-Serine (Cfinal = 400 μM), L-Homocysteine (Cfinal = 400 μM), 5,5'-dithio bis-(2-nitrobenzoic acid) (DTNB; Cfinal = 150 μM). After incubation for 60 min at 37°C, the absorbance change was measured at 420 nm (ΔOD420). For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (L-homocysteine and L-serine).

Reference:

Y. Zhou et al., High-throughput tandem-microwell assay identifies inhibitors of the hydrogen sulfide signaling pathway. Chem Commun (Camb) 49, 11782-11784 (2013).

1. **Carboxypeptidase U (EC: 3.4.17.20)**

Reaction:

Release of C-terminal Arg and Lys from a polypeptide.

Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and the reaction was started by the addition of substrate (Hippuryl-Arg carboxypeptidase substrate, Cfinal = 1 mM). After incubation for 30 min at 37°C, the absorbance was measured at 254 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (Hippuryl-Arg carboxypeptidase substrate).

Reference:

D. V. Marinkovic, J. N. Marinkovic, E. G. Erdös, C. J. Robinson, Purification of carboxypeptidase B from human pancreas. Biochem J 163, 253-260 (1977).

1. **Purine-nucleoside phosphorylase (EC: 2.4.2.1)**

Reaction:

guanosine + phosphate = alpha-D-ribose 1-phosphate + guanine



Reaction system: Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing guanosine (Cfinal = 400 μM). After incubation for 30 min at 25°C, and the absorbance was measured at 258 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (guanosine).

Reference:

B. K. Kim, S. Cha, R. E. Parks, Jr., Purine nucleoside phosphorylase from human erythrocytes. I. Purification and properties. J Biol Chem 243, 1763-1770 (1968).

1. **Aromatase (****EC: 1.14.14.14)**

Reaction:

3 O2 + 3 FMNH2 + testosterone = 17beta-estradiol + formate + 4 H+ + 4 H2O + 3 FMN



Reaction system: 100 μL Secreted protein extracting solution (Cfinal = 10 μg/mL) and substrate mixture (50 μg/mL of protein and 0.4 μM dibenzylfluorescein (DBF)) was added to 100 μL 50 mM potassium phosphate buffer containing 2.6 mM NADP+, 7.6 mM glucose 6-phosphate, 0.8 U/mL glucose 6-phosphate dehydrogenase, 13.9 mM MgCl2, and 1 mg/mL albumin. After incubation for 30 min at 37°C, solution was quenched with 75 μL of 2 M NaOH, shaken for 5 min, and incubated for 2 h at 37°C. Finally, fluorescence was measured at 485 nm (excitation) and 530 nm (emission). For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (DBF).

Reference:

D. C. Endringer, K. G. Guimarães, T. P. Kondratyuk, J. M. Pezzuto, F. C. Braga, Selective inhibition of aromatase by a dihydroisocoumarin from Xyris pterygoblephara. J Nat Prod 71, 1082-1084 (2008).

1. **Arachidonate 5-lipoxygenase (EC: 1.13.11.34)**

Reaction:

(5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate + O2 = (8*S*)-hydroperoxy-(5*Z*,9*E*,11*Z*,14*Z*)-eicosatetraenoate



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to 50 mM Tris-HCl buffer, containing ATP (Cfinal = 0.2 mM), dithiothreitol (Cfinal = 0.1 mM), EDTA (Cfinal = 0.1 mM), CaCl2 (Cfinal = 0.5 mM) and H2DCFDA (Cfinal = 10 μM), and the reaction was started by the addition of arachidonic acid (Cfinal = 25 μM). After incubation for 30 min at 37°C, then monitored by excitation at 500 nm and emission at 520 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (arachidonic acid).

Reference:

Y. Wu et al., Dynamic modeling of human 5-lipoxygenase-inhibitor interactions helps to discover novel inhibitors. J Med Chem 55, 2597-2605 (2012).

1. **Amino-acid N-acetyltransferase (EC: 2.3.1.1)**

Reaction:

acetyl-CoA + L-glutamate = CoA + H+ + N-acetyl-L-glutamate



Reaction system: Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing glutamate (Cfinal = 3 mM), acetyl-CoA (Cfinal = 400 μM), DTNB (Cfinal = 300 μM). After incubation for 60 min at 37°C, the absorbance change was measured at 420 nm (ΔOD420). For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (L-glutamate).

Reference:

E. Sancho-Vaello, M. L. Fernández-Murga, V. Rubio, Functional dissection of N-acetylglutamate synthase (ArgA) of Pseudomonas aeruginosa and restoration of its ancestral N-acetylglutamate kinase activity. J Bacteriol 194, 2791-2801 (2012).

1. **Adenosine deaminase (EC: 3.5.4.4)**

Reaction:

adenosine + H+ + H2O = inosine + NH4+



Reaction system: Based on the protocol of the detection kit (Biotides Cat# TE0233). Briefly, secreted protein extracting solution (Cfinal = 10 μg/mL) was added to Good's buffer and reaction with adenosine to generate inosine and ammonia, and the generated ammonia was further reacted with pypocholoride and phenol to generate water soluble indophenol blue. After incubation for 30 min at 37°C, the absorbance was measured at 630 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (adenosine).

Reference:

Z. Namiot et al., Adenosine deaminase activity in patients with the intestinal type of gastric carcinoma. Cancer Lett 109, 199-202 (1996).

1. **Gamma-glutamyl hydrolase (EC: 3.4.19.9)**

Reaction:

(6S)-5,6,7,8-tetrahydrofolyl-(gamma-L-Glu) (n) + (n-1) H2O = (6S)-5,6,7,8-tetrahydrofolate + (n-1) L-glutamate



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and reacted by the addition of 200 μM H-Glu-pNA. After incubation for 30 min at 37°C, and the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (H-Glu-pNA).

Reference:

M. A. Durá, M. Flores, F. Toldrá, Purification and characterisation of a glutaminase from Debaryomyces spp. *Int J Food Microbiol* **76**, 117-126 (2002).

1. **Kynureninase (EC: 3.7.1.3)**

Reaction:

H2O + L-kynurenine = anthranilate + H+ + L-alanine



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing MgSO4 (Cfinal = 2 mM), pyridoxal phosphate (PLP; Cfinal = 4 μM) and reacted by the addition of kynurenine (Cfinal = 400 μM). After incubation for 30 min at 37°C, then monitored by excitation at 315 nm and emission at 400 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (kynurenine).

Reference:

F. H. Gaertner, K. W. Cole, G. R. Welch, Evidence for distinct kynureninase and hydroxykynureninase activities in Neurospora crassa. *J Bacteriol* **108**, 902-909 (1971).

1. **Soluble epoxide hydrolase (EC: 3.3.2.10)**

Reaction:

an epoxide + H2O = an ethanediol



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing PHOME (Cfinal = 10 μM). After incubation for 30 min at 37°C, then monitored by excitation at 330 nm and emission at 460 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (PHOME).

Reference:

G. Abis, R. L. Charles, P. Eaton, M. R. Conte, Expression, purification, and characterisation of human soluble Epoxide Hydrolase (hsEH) and of its functional C-terminal domain. *Protein Expr Purif* **153**, 105-113 (2019).

1. **Memapsin 2 (EC: 3.4.23.46)**

Reaction:

Broad endopeptidase specificity. Cleaves Glu-Val-Asn-Leu-|-Asp-Ala-Glu-Phe in the Swedish variant of Alzheimer's amyloid precursor protein.

Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and the reaction was started by the addition of N-Benzoyl-DL-arginine-p-nitroaniline (BAPNA; Cfinal = 3 mM). After incubation for 30 min at 37°C, the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (BAPNA).

Reference:

F. Mancini, M. Naldi, V. Cavrini, V. Andrisano, Multiwell fluorometric and colorimetric microassays for the evaluation of beta-secretase (BACE-1) inhibitors. *Anal Bioanal Chem* **388**, 1175-1183 (2007).

1. **Cathepsin B (EC: 3.4.22.1)**

Reaction:

Hydrolysis of proteins with broad specificity for peptide bonds. Preferentially cleaves -Arg-Arg-|-Xaa bonds in small molecule substrates. In addition to being an endopeptidase, shows peptidyl-dipeptidase activity, liberating C-terminal dipeptides.

Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and the reaction was started by the addition of substrate (Z-Phe-Arg-pNA, Cfinal = 500 μM). After incubation for 30 min at 37°C, the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (Z-Phe-Arg-pNA).

Reference:

G. Z. Zeng, X. L. Pan, N. H. Tan, J. Xiong, Y. M. Zhang, Natural biflavones as novel inhibitors of cathepsin B and K. *Eur J Med Chem* **41**, 1247-1252 (2006).

1. **Triacylglycerol lipase (EC: 3.1.1.3)**

Reaction:

a triacylglycerol + H2O = a diacylglycerol + a fatty acid + H+



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and the reaction was started by the addition of P-nitrophenol palmitate (Cfinal = 200 μM). After incubation for 30 min at 37°C, the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (P-nitrophenol palmitate).

Reference:

X. L. Qiu, Q. F. Zhang, Chemical profile and pancreatic lipase inhibitory activity of Sinobambusa tootsik (Sieb.) Makino leaves. *PeerJ* **7**, e7765 (2019).

1. **Uridine phosphorylase (EC: 2.4.2.3)**

Reaction:

phosphate + uridine = alpha-D-ribose 1-phosphate + uracil



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and reacted by the addition of uridine (Cfinal = 5mM). After incubation for 5 min at 37°C, and the absorbance was measured at 280 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (uridine).

Reference:

D. Renck *et al.*, Design of novel potent inhibitors of human uridine phosphorylase-1: synthesis, inhibition studies, thermodynamics, and in vitro influence on 5-fluorouracil cytotoxicity. *J Med Chem* **56**, 8892-8902 (2013).

1. **Acetylcholinesterase (EC: 3.1.1.7)**

Reaction:

acetylcholine + H2O = acetate + choline + H+



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and the reaction was started by the addition of acetylthiocholine iodide (ASCH; Cfinal = 500 μM), and DTNB (Cfinal = 500 μM). After incubation for 30 min at 37°C, the absorbance was measured at 420 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (acetylthiocholine iodide).

Reference:

M. Pohanka, M. Hrabinova, K. Kuca, J. P. Simonato, Assessment of acetylcholinesterase activity using indoxylacetate and comparison with the standard Ellman's method. *Int J Mol Sci* **12**, 2631-2640 (2011).

1. **Carbonic anhydrase (EC: 4.2.1.1)**

Reaction:

H+ + hydrogencarbonate = CO2 + H2O



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing nitrophenylacetate (Cfinal = 3 mM). After incubation for 30 min at 25°C, the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (nitrophenylacetate).

Reference:

B. P. Ko, A. Yazgan, P. L. Yeagle, S. C. Lottich, R. W. Henkens, Kinetics and mechanism of refolding of bovine carbonic anhydrase. A probe study of the formation of the active site. *Biochemistry* **16**, 1720-1725 (1977).

1. **Ornithine aminotransferase (EC: 2.6.1.13)**

Reaction:

2-oxoglutarate + L-ornithine = L-glutamate + L-glutamate 5-semialdehyde



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to 100 μL PBS buffer containing PLP (Cfinal = 20 μM), 2-oxoglutarate (Cfinal = 20 mM), ornithine (Cfinal = 60 mM), incubation for 30 min at 37°C and the reaction was stopped by the addition of 100 μL 10 % trichloroacetic acid in water and 200 μL 5% o-amino benzaldehyde in ethanol. After incubation for 150 min at 37°C, the absorbance was measured at 440 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (2-oxoglutarate and ornithine).

Reference:

M. J. Jung, N. Seiler, Enzyme-activated irreversible inhibitors of L-ornithine:2-oxoacid aminotransferase. Demonstration of mechanistic features of the inhibition of ornithine aminotransferase by 4-aminohex-5-ynoic acid and gabaculine and correlation with in vivo activity. *J Biol Chem* **253**, 7431-7439 (1978).

1. **Alpha-amylase (****EC:** **3.2.1.1)**

Reaction:

Catalyzes the hydrolysis of internal (1->4)-alpha-D-glucosidic bonds, yielding a mixture of maltose, isomaltose, small amounts of glucose as well as small linear and branched oligosaccharides called dextrins.

Reaction system: Based on the protocol of the detection kit (Solarbio Cat# BC0610). Briefly, secreted protein extracting solution (Cfinal = 10 μg/mL) was adding amylum and incubation for 10 min at 37°C to generate reducing sugar, and the generated reducing sugar was further reacted with 3,5- dinitrosalicylic acid. After incubation for 10 min at 37°C, the absorbance was measured at 540 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (amylum).

Reference:

S. Lakshmana Senthil *et al.*, Fucoidan - An α-amylase inhibitor from Sargassum wightii with relevance to NIDDM. *Int J Biol Macromol* **81**, 644-647 (2015).

1. **Thymidine phosphorylase (EC 2.4.2.4)**

Reaction:

phosphate + thymidine = thymine + 2-deoxy-alpha-D-ribose 1-phosphate



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing thymidine-5'-monophosphate disodium salt (Cfinal = 1.5 mM). After incubation for 30 min at 37°C, the absorbance was measured at 290 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (thymidine).

Reference:

K. Zaman *et al.*, Synthesis, thymidine phosphorylase, angiogenic inhibition and molecular docking study of isoquinoline derivatives. *Bioorg Chem* **89**, 102999 (2019).

1. **Alpha-galactosidase (EC 3.2.1.22)**

Reaction:

globoside Gb3Cer (d18:1(4E)) + H2O = a beta-D-Gal-(1->4)-beta-D-Glc-(1<->1)-Cer(d18:1(4E)) + D-galactose



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing X-α-gal (Cfinal = 100 μM). After incubation for 30 min at 37°C, the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (X-α-gal).

Refernce: D. A. Post, V. E. Luebke, Purification, cloning, and properties of alpha-galactosidase from *Saccharopolyspora erythraea* and its use as a reporter system. *Appl Microbiol Biotechnol* **67**, 91-96 (2005).

1. **7-dehydrocholesterol reductase (EC 1.3.1.21)**

Reaction:

7-dehydrocholesterol + H+ + NADPH = cholesterol + NADP+



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing 7-dehydrocholesterol (Cfinal = 100 μM), and NADPH (Cfinal = 100 μM). After incubation for 30 min at 37°C, the cholesterol was measured by HPLC. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (7-dehydrocholesterol).

Reference:

H. Nishino, T. Ishibashi, Evidence for requirement of NADPH-cytochrome P450 oxidoreductase in the microsomal NADPH-sterol Delta7-reductase system. *Arch Biochem Biophys* **374**, 293-298 (2000).

1. **GMP reductase (****EC 1.7.1.7)**

Reaction:

GMP + 2 H+ + NADPH = IMP + NADP+ + NH4+



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and reacted by the addition of NADPH (Cfinal = 100 μM), GMP (Cfinal = 100 μM). After incubation for 30 min at 25°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (GMP).

Reference:

L. K. Martinelli *et al.*, Recombinant Escherichia coli GMP reductase: kinetic, catalytic and chemical mechanisms, and thermodynamics of enzyme-ligand binary complex formation. *Mol Biosyst* **7**, 1289-1305 (2011).

1. **Asparaginase (EC 3.5.1.1)**

Reaction:

H2O + L-asparagine = L-aspartate + NH4+



Reaction system: Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing L-asparagine (Cfinal = 4 mM). After incubation for 10 min at 45°C, added Nessler reagent and the absorbance was measured at 420 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (L-asparagine).

Reference:

Y. Zhang, D. Li, Y. Li, Expression and purification of L-asparaginase from Escherichia coli and the inhibitory effects of cyclic dipeptides. *Nat Prod Res* **31**, 2099-2106 (2017).

1. **Aldehyde dehydrogenase (NAD (+)) (EC 1.2.1.3)**

Reaction:

an aldehyde + H2O + NAD+ = a carboxylate + 2 H+ + NADH



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and reacted by the addition of acetaldehyde (Cfinal = 1.5 mM) and NAD+ (Cfinal = 1 mM). After incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (acetaldehyde).

Reference:

B. Parajuli *et al.*, Discovery of novel regulators of aldehyde dehydrogenase isoenzymes. *Chem Biol Interact* **191**, 153-158 (2011).

1. **Nicotinamide N-methytransferase (EC** **2.1.1.1)**

Reaction:

nicotinamide + S-adenosyl-L-methionine = 1-methylnicotinamide + S-adenosyl-L-homocysteine



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to 80 μL Tris buffer (100 mM Tris Hcl pH 7.5, 0.04% BSA, 2 mM Dithiothreitol, 20 μM formic acid and 1% DMSO) containing nicotinamide (Cfinal = 20 μM), SAM (Cfinal = 20 μM), and the reaction was started by the addition of 10 μL 50% acetophenone in alcohol and 10 μL 5 μM KOH in PBS buffer. After incubation for 30 min at 37°C, then monitored by excitation at 375 nm and emission at 430 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Note: the chromogenic reaction was based on the generated 1-methylnicotinamide, which reacts with acetophenone in the presence of KOH and formic acid and forms a fluorescent product, 2, 7-naphthyridine.

Substrate: natural substrate (Nicotinamide).

Reference:

S. Ruf et al., Novel nicotinamide analog as inhibitor of nicotinamide N-methyltransferase. Bioorg Med Chem Lett 28, 922-925 (2018).

1. **Tryptophan 5-monooxygenase (****EC 1.14.16.4)**

Reaction:

(6R)-L-erythro-5,6,7,8-tetrahydrobiopterin + L-tryptophan + O2 = (4aS,6R)-4a-hydroxy-L-erythro-5,6,7,8-tetrahydrobiopterin + 5-hydroxy-L-tryptophan



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to buffer containing tryptophan (Cfinal = 60 μM), 6-methyl tetrahydrotrexate (Cfinal = 300 μM), ammonium sulfate (Cfinal = 200 mM), DTT (Cfinal = 7 mM), catalase (Cfinal = 25 μg/ml), ferrous (Cfinal = 25 μm), and Mes (Cfinal = 50 mM). After incubation for 30 min at 37°C, then monitored by excitation at 300 nm and emission at 330 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (tryptophan).

Reference:

H. Shi, Y. Cui, Y. Qin, Discovery and characterization of a novel tryptophan hydroxylase 1 inhibitor as a prodrug. *Chem Biol Drug Des* **91**, 202-212 (2018).

1. **Methionyl aminopeptidase (EC 3.4.11.18)**

Reaction:

Release of N-terminal amino acids, preferentially methionine, from peptides and arylamides.

Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and the reaction was started by the addition of Met-pNA (Cfinal = 1 mM). After incubation for 30 min at 37°C, the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (Met-pNA).

Reference:

M. Huang *et al.*, Inhibition of monometalated methionine aminopeptidase: inhibitor discovery and crystallographic analysis. *J Med Chem* **50**, 5735-5742 (2007).

1. **15-hydroxyprostaglandin dehydrogenase (EC 1.1.1.141)**

Reaction:

NAD+ + prostaglandin E2 = 15-oxoprostaglandin E2 + H+ + NADH



Reaction system: Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing PGE2 (Cfinal = 25 μM), NAD+ (Cfinal = 200 μM). After incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (PGE2).

Reference:

S. Karna, In-vitro Wound Healing Effect of 15-Hydroxyprostaglandin Dehydrogenase Inhibitor from Plant. *Pharmacogn Mag* **13**, S122-s126 (2017).

1. **Phosphoserine transaminase (EC 2.6.1.52)**

Reaction:

2-oxoglutarate + O-phospho-L-serine = 3-phosphooxypyruvate + L-glutamate



Reaction system: Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing O-phospho-L-serine (Cfinal = 3 mM), 2-oxoglutarate (Cfinal = 3 mM), and glutamate dehydrogenase (Cfinal = 90 U), and adding NAD+ (Cfinal = 2 mM). After incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (O-phospho-L-serine and 2-oxoglutarate).

Reference:

V. Ali, T. Nozaki, Biochemical and functional characterization of phosphoserine aminotransferase from Entamoeba histolytica, which possesses both phosphorylated and non-phosphorylated serine metabolic pathways. *Mol Biochem Parasitol* **145**, 71-83 (2006).

1. **Trypsin (EC 3.4.21.4)**

Reaction:

Preferential cleavage: Arg-|-Xaa, Lys-|-Xaa.

Reaction system: Based on the protocol of the detection kit (Solarbio Cat# BC2310). Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to reaction buffer and reaction with N-benzoyl-L-arginine-ethylester (BAEE), and incubation for 30 min at 37°C to generate N-Benzoyl-L-Arginine, the absorbance was measured at 253 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (BAEE).

Reference:

J. Qian *et al.*, Study the effect of trypsin enzyme activity on the screening of applying frontal affinity chromatography. *Int J Biol Macromol* **139**, 740-751 (2019).

1. **Ceramide glucosyltransferase (****EC 2.4.1.80)**

Reaction:

an N-acylsphing-4-enine + UDP-alpha-D-glucose =

a beta-D-glucosyl-(1<->1')-N-acylsphing-4-enine + UDP



Reaction system: Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing UDP-Glc (Cfinal = 500 mM), and equivalent liposomes containing C16-Cer (0.05 mg/mL) and lecithin (0.5 mg/mL). After incubation for 1 hour at 37°C, the remaining C16-Cer was measured by LC-MS/MS method. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (C16-Cer).

Reference:

S. Ichikawa, H. Sakiyama, G. Suzuki, K. I. Hidari, Y. Hirabayashi, Expression cloning of a cDNA for human ceramide glucosyltransferase that catalyzes the first glycosylation step of glycosphingolipid synthesis. *Proc Natl Acad Sci U S A* **93**, 4638-4643 (1996).

1. **Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)**

Reaction:

D-glucose 6-phosphate + NADP+ = 6-phospho-D-glucono-1,5-lactone + H+ + NADPH



Reaction system: Based on the protocol of the detection kit (Solarbio Cat# BC0260). Secreted protein extracting solution (final concentration at 10 μg/mL) was added to reaction buffer and reaction with D-glucose 6-phosphate and NADP+, after incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (D-glucose 6-phosphate).

Reference:

L. Kanwal, S. Ali, A. Rasul, H. M. Tahir, Smilax china root extract as a novel Glucose- 6-phosphate dehydrogenase inhibitor for the treatment of hepatocellular carcinoma. *Saudi J Biol Sci* **29**, 103400 (2022).

1. **17beta-estradiol 17-dehydrogenase (EC 1.1.1.62)**

Reaction:

17beta-estradiol + NAD+ = estrone + H+ + NADH



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and reacted by the addition of 200 μM 17β-Estradiol, and 500 μM NAD+. After incubation for 30 min at 37°C, and the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (17β-Estradiol).

Reference:

X. Ye *et al.*, A novel 17β-hydroxysteroid dehydrogenase in Rhodococcus sp. P14 for transforming 17β-estradiol to estrone. *Chem Biol Interact* **276**, 105-112 (2017).

1. **Dihydropyrimidinase (EC: 3.5.2.2)**

Reaction:

5,6-dihydrouracil + H2O = 3-(carbamoylamino)propanoate + H+



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and reacted by the addition of 1 mM dihydrouracil. After incubation for 30 min at 37°C, and the absorbance was measured at 230 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (dihydrouracil).

Reference:

C. Y. Huang, Inhibition of a Putative Dihydropyrimidinase from Pseudomonas aeruginosa PAO1 by Flavonoids and Substrates of Cyclic Amidohydrolases. *PLoS One* 10, e0127634 (2015).

1. **Granzyme B (EC 3.4.21.79)**

Reaction:

Preferential cleavage: -Asp-|-Xaa- >> -Asn-|-Xaa- > -Met-|-Xaa-, -Ser-|-Xaa-.

Reaction system: Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing Ac-IEPD-pNA (Cfinal = 200 μM). After incubation for 30 min at 37°C, and the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (Ac-IEPD-pNA).

Reference:

M. Marcet-Palacios et al., Design and characterization of a novel human Granzyme B inhibitor. *Protein Eng Des Sel* 28, 9-17 (2015).

1. **Adenosylhomocysteinase (EC 3.13.2.1)**

Reaction:

H2O + S-adenosyl-L-homocysteine = adenosine + L-homocysteine



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing SAM (Cfinal = 500 μM), and DTNB (Cfinal = 150 μM). After incubation for 30 min at 37°C, the absorbance was measured at 420 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (SAM).

Reference:

C. S. Yuan, D. B. Ault-Riché, R. T. Borchardt, Chemical modification and site-directed mutagenesis of cysteine residues in human placental S-adenosylhomocysteine hydrolase. *J Biol Chem* 271, 28009-28016 (1996).

1. **Gamma-glutamyltransferase (EC 2.3.2.2)**

Reaction:

an alpha-amino acid + an N-terminal (5-L-glutamyl)-[peptide] = 5-L-glutamyl amino acid + N-terminal L-alpha-aminoacyl-[peptide]



Reaction system: Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer containing H-Glu-PNA (Cfinal = 200 μM) and H-Gly-Gly-OMe (Cfinal = 200 μM). After incubation for 30 min at 37°C, and the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (H-Glu-PNA and H-Gly-Gly-OMe).

Reference:

M. Franzini *et al.*, Gamma-glutamyltransferase activity in human atherosclerotic plaques--biochemical similarities with the circulating enzyme. *Atherosclerosis* **202**, 119-127 (2009).

1. **Thioredoxin-dependent peroxiredoxin (EC 1.11.1.24)**

Reaction:

[thioredoxin]-dithiol + a hydroperoxide = [thioredoxin]-disulfide + an alcohol + H2O



Reaction system: Based on the protocol of the detection kit (Solarbio Cat# BC0090). Secreted protein extracting solution (final concentration at 10 μg/mL) was added to reaction buffer and reaction with hydrogen peroxide and oxidation substrate, after incubation for 30 min at 37°C, the absorbance was measured at 470 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (hydrogen peroxide).

Reference:

J. Liao *et al.*, Cloning, expression and antioxidant activity of a thioredoxin peroxidase from Branchiostoma belcheri tsingtaunese. *PLoS One* **12**, e0175162 (2017).

1. **GTP cyclohydrolase I (EC: 3.5.4.16)**

Reaction:

GTP + H2O = 7,8-dihydroneopterin 3'-triphosphate + formate + H+



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and reacted by the addition of 200 μM GTP. After incubation for 30 min at 37°C, and the absorbance was measured at 330 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (GTP).

Reference:

S. Schüssler et al., Structure of GTP cyclohydrolase I from Listeria monocytogenes, a potential anti-infective drug target. *Acta Crystallogr F Struct Biol Commun* 75, 586-592 (2019).

1. **Porphobilinogen synthase (EC 4.2.1.24)**

Reaction:

2 5-aminolevulinate = H+ + 2 H2O + porphobilinogen



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to 100 μL Tris buffer (100 mM Tris, 5 mM DTT, 1 mM MgCl2) containing 5-aminolevulinate (Cfinal = 200 μM). After incubation for 30 min at 25°C, the reaction was stopped with 50 μL modified Ehrlich’s Reagent (1 g 4-dimethylamino benzaldehyde in 42 mL acetic acid, 12 mL perchloric acid, 7.3 mL 12% trichloroacetic acid), and the absorbance was measured at 555 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (5-aminolevulinate).

Reference:

C. S. Lentz et al., New chemotypes for wALADin1-like inhibitors of delta-aminolevulinic acid dehydratase from Wolbachia endobacteria. *Bioorg Med Chem Lett* 23, 5558-5562 (2013).

1. **Alcohol dehydrogenase (EC 1.1.1.1)**

Reaction:

a primary alcohol + NAD+ = an aldehyde + H+ + NADH



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and reacted by the addition of alcohol (Cfinal = 20 mM) and NAD+ (Cfinal = 1 mM). After incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (alcohol).

Reference:

Y. Wuxiuer *et al.*, An intact eight-membered water chain in drosophilid alcohol dehydrogenases is essential for optimal enzyme activity. *Febs j* **279**, 2940-2956 (2012).

1. **Glutathione-disulfide reductase (EC 1.8.1.7)**

Reaction:

glutathione disulfide + H+ + NADPH = 2 glutathione + NADP+



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and reacted by the addition of glutathione disulfide (Cfinal = 1 mM), NADPH (Cfinal = 1 mM). After incubation for 30 min at 25°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (glutathione disulfide).

Reference:

B. Mannervik, Measurement of glutathione reductase activity. Curr Protoc Toxicol Chapter 7, Unit7.2 (2001).

1. **Hyaluronoglucosaminidase (EC 3.2.1.35)**

Reaction:

Random hydrolysis of (1->4)-linkages between N-acetyl-beta-D-glucosamine and D-glucuronate residues in hyaluronate.

Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to 80 μL PBS buffer (with 0.1% sodium hyaluronate) and incubation for 60 min at 37°C. Then reacted by the addition of 1 mL 2.5% cetyltrimethylammonium bromide and 2% NaOH in water. After incubation for 30 min at 25°C, the absorbance was measured at 400 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (hyaluronate).

Reference:

E. Harunari et al., Hyaluromycin, a new hyaluronidase inhibitor of polyketide origin from marine Streptomyces sp. *Mar Drugs 12*, 491-507 (2014).

1. **L-iditol 2-dehydrogenase (****EC** **1.1.1.14)**

Reaction:

D-sorbitol + NAD+ = + keto-D-fructose + NADH + H+



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and reacted by the addition of D-Sorbitol (Cfinal = 200 μM), NAD+ (Cfinal = 200 μM). After incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (D-Sorbitol).

Reference:

R. I. Lindstad, K. Teigen, L. Skjeldal, Inhibition of sorbitol dehydrogenase by nucleosides and nucleotides. *Biochem Biophys Res Commun* 435, 202-208 (2013).

1. **Glutamine synthetase (EC 6.3.1.2)**

Reaction:

ATP + L-glutamate + NH4+ = ADP + H+ + L-glutamine + phosphate



Reaction system: Based on the protocol of the detection kit (Solarbio Cat# BC0910). Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to reaction buffer and reaction with L-glutamate to generate L-glutamine, and then reacted to γ-glutamyl hydroxamic acid, which forming red complex with iron. After incubation for 30 min at 37°C, the absorbance was measured at 540 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (L-glutamate).

Reference:

R. Minet, F. Villie, M. Marcollet, D. Meynial-Denis, L. Cynober, Measurement of glutamine synthetase activity in rat muscle by a colorimetric assay. *Clin Chim Acta* **268**, 121-132 (1997).

1. **Caspase-1 (EC 3.4.22.36)**

Reaction:

Strict requirement for an Asp residue at position P1 and has a preferred cleavage sequence of Tyr-Val-Ala-Asp-|-.

Reaction system: Based on the protocol of the detection kit (Solarbio Cat# BC3810). Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to reaction buffer and reaction with Tyr-Val-Ala-Asp-p-nitroanilide (YVAD-pNA), after incubation for 30 min at 37°C, the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (YVAD-pNA).

Reference:

F. Ulgheri *et al.*, Design, synthesis and biological evaluation of 1,5-disubstituted α-amino tetrazole derivatives as non-covalent inflammasome-caspase-1 complex inhibitors with potential application against immune and inflammatory disorders. *Eur J Med Chem* **229**, 114002 (2022).

1. **Glycine N-acyltransferase (EC 2.3.1.13)**

Reaction:

an acyl-CoA + glycine = an N-acylglycine + CoA + H+



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and reacted by the addition of acetyl-CoA (Cfinal = 500 μM), glycine (Cfinal = 200 mM), and DTNB (Cfinal = 300 μM). After incubation for 30 min at 37°C, adjust the pH value to 13 and the absorbance was measured at 420 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (glycine).

Reference:

R. van der Sluis, C. P. Badenhorst, F. H. van der Westhuizen, A. A. van Dijk, Characterisation of the influence of genetic variations on the enzyme activity of a recombinant human glycine N-acyltransferase. *Gene* **515**, 447-453 (2013).

1. **Glucose-6-phosphate isomerase (EC 5.3.1.9)**

Reaction:

beta-D-fructose 6-phosphate = alpha-D-glucose 6-phosphate



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and reacted by the addition of F6P (Cfinal = 2 mM), NAD+ (Cfinal = 200 μM), and G6PDH (2 U). After incubation for 30 min at 37°C the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (F6P).

Reference:

R. Eltahan, F. Guo, H. Zhang, L. Xiang, G. Zhu, Discovery of ebselen as an inhibitor of Cryptosporidium parvum glucose-6-phosphate isomerase (CpGPI) by high-throughput screening of existing drugs. *Int J Parasitol Drugs Drug Resist* 8, 43-49 (2018).

1. **L-xylulose reductase (EC 1.1.1.10)**

Reaction:

H+ + L-xylulose + NADPH = NADP+ + xylitol



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to 0.1M potassium phosphate buffer, and reacted by the addition of 5mM diacetyl, 100 μM L-xylulose, and 100 μM NADPH. After incubation for 30 min at 37°C the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (L-xylulose).

Reference:

V. Carbone, S. Ishikura, A. Hara, O. El-Kabbani, Structure-based discovery of human L-xylulose reductase inhibitors from database screening and molecular docking. Bioorg Med Chem 13, 301-312 (2005).

1. **Aldose reductase (EC 1.1.1.21)**

Reaction:

an aldose + H+ + NADPH = an alditol + NADP+



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and reacted by the addition of NADPH (Cfinal = 160 μM), and DL-glyceraldehyde (Cfinal = 10 mM). After incubation for 30 min at 37°C the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (DL-glyceraldehyde).

Reference:

L. Ji et al., A simple and stable galactosemic cataract model for rats. *Int J Clin Exp Med* 8, 12874-12881 (2015).

1. **5’-nucleotidase (EC 3.1.3.5)**

Reaction:

a ribonucleoside 5'-phosphate + H2O = a ribonucleoside + phosphate



Reaction system: Based on the protocol of the detection kit (Solarbio Cat# BC4590). Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to reaction buffer and reaction with ribonucleoside 5’-phosphate, after incubation for 30 min at 37°C, the generated phosphate was detected by phosphorus chromogenic method, the absorbance was measured at 660 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (ribonucleoside 5’-phosphate).

Reference:

J. B. De Jesus, D. Cosentino-Gomes, J. R. Meyer-Fernandes, Characterization of an ecto-5'-nucleotidase activity present on the cell surface of Tritrichomonas foetus. *Vet Parasitol* **179**, 50-56 (2011).

1. **Tyrosine 3-monooxygenase (EC: 1.14.16.2)**

Reaction:

(6R)-L-erythro-5,6,7,8-tetrahydrobiopterin + L-tyrosine + O2 = (4aS,6R)-4a-hydroxy-L-erythro-5,6,7,8-tetrahydrobiopterin + L-dopa



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer (containing 500 μM FeSO4), and reacted by the addition of 200 μM tyrosine, 250 μM tetrahydrobiopterin, and 200 μM NaIO4. After incubation for 30 min at 37°C, the absorbance was measured at 475 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (tyrosine and tetrahydrobiopterin).

Reference:

Y. Wang, C. C. Sung, K. K. Chung, Novel enhancement mechanism of tyrosine hydroxylase enzymatic activity by nitric oxide through S-nitrosylation. *Sci Rep* 7, 44154 (2017).

1. **L-lactate dehydrogenase (EC 1.1.1.27)**

Reaction:

(S)-lactate + NAD+ = H+ + NADH + pyruvate



Reaction system: Based on the protocol of the detection kit (Solarbio Cat# BC0680). Secreted protein extracting solution (final concentration at 10 μg/mL) was added to reaction buffer and reaction with (S)-lactate, and incubation for 30 min at 37°C to produce pyruvate, which further reacted with 2,4- dinitro-phenylhydrazine to generate brownish red products. The absorbance was measured at 450 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate ((S)-lactate).

Reference:

P. Chandra, K. R. Hegde, S. D. Varma, Possibility of topical antioxidant treatment of cataracts: corneal penetration of pyruvate in humans. *Ophthalmologica* **223**, 136-138 (2009).

1. **Proline dehydrogenase (EC 1.5.5.2)**

Reaction:

a quinone + L-proline = (S)-1-pyrroline-5-carboxylate + a quinol + H+



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and reacted by the addition of Cytochrome C (Cfinal = 60 μM), KCN (Cfinal = 1mM), and L-proline (Cfinal = 100 mM). After incubation for 30 min at 37°C the absorbance was measured at 550 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (L-proline).

Reference:

P. Schertl et al., Biochemical characterization of proline dehydrogenase in Arabidopsis mitochondria. *Febs j* 281, 2794-2804 (2014).

1. **Protein farnesyltransferase (EC: 2.5.1.58)**

Reaction:

(2E,6E)-farnesyl diphosphate + L-cysteinyl-[protein] = diphosphate + S-(2E,6E)-farnesyl-L-cysteinyl-[protein]



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer (5 mM DTT, 5 mM MgCl2, 10 μM ZnCl2, 0.2% octyl 3-D-glucopyranoside), and reacted by the addition of 1 μM N-dansyl-GCVLS, 5 μM farnesyl diphosphate. After incubation for 30 min at 37°C, then monitored by excitation at 340 nm and emission at 505 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (farnesyl diphosphate).

Reference:

D. L. Pompliano, R. P. Gomez, N. J. Anthony, Intramolecular fluorescence enhancement: a continuous assay of Ras farnesyl:protein transferase. *Journal of the American Chemical Society* 114, 7945-7946 (1992).

1. **Xanthine dehydrogenase (EC: 1.17.1.4)**

Reaction:

H2O + NAD+ + xanthine = H+ + NADH + urate



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and reacted by the addition of 300 μM Xanthine. After incubation for 10 min at 37°C, and the absorbance was measured at 295 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (Xanthine).

Reference:

R. Yu *et al.*, Oxanosine Monophosphate Is a Covalent Inhibitor of Inosine 5'-Monophosphate Dehydrogenase. *Chem Res Toxicol* **32**, 456-466 (2019).

1. **Catalase (EC: 1.11.1.6)**

Reaction:

2 H2O2 = 2 H2O + O2



Reaction system: Based on the protocol of the detection kit (Solarbio Cat# BC0200). Secreted protein extracting solution (final concentration at 10 μg/mL) was added to reaction buffer and reaction with hydrogen peroxide, after incubation for 30 min at 37°C, the absorbance was measured at 240 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (hydrogen peroxide).

Reference:

J. Krych-Madej, L. Gebicka, Interactions of nitrite with catalase: Enzyme activity and reaction kinetics studies. *J Inorg Biochem* **171**, 10-17 (2017).

1. **IMP dehydrogenase (EC: 1.1.1.205)**

Reaction:

H2O + IMP + NAD+ = H+ + NADH + XMP



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and reacted by the addition of 200 μM IMP, and 200 μM NAD+. After incubation for 30 min at 37°C, and the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (IMP).

Reference:

R. Yu et al., Oxanosine Monophosphate Is a Covalent Inhibitor of Inosine 5'-Monophosphate Dehydrogenase. *Chem Res Toxicol* 32, 456-466 (2019).

1. **Adenylyl cyclase (EC: 4.6.1.1)**

Reaction:

ATP = 3',5'-cyclic AMP + diphosphate



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and reacted by the addition of 10 μM ATP. After incubation for 30 min at 37°C, the remaining ATP was measured by ATP detection kit. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (ATP).

Reference:

A. S. Sikarwar *et al.*, Hypoxia inhibits adenylyl cyclase catalytic activity in a porcine model of persistent pulmonary hypertension of the newborn. *Am J Physiol Lung Cell Mol Physiol* **315**, L933-l944 (2018).

1. **Cerebroside-sulfatase (EC 3.1.6.8)**

Reaction:

H2O + N-acyl-1-beta-D-(3-O-sulfo)-galactosyl-sphing-4-enine =

a beta-D-galactosyl-(1<->1')-N-acylsphing-4-enine + H+ + sulfate



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, and reacted by the addition of 4-methylumbelliferyl sulfate potassium salt (Cfinal = 500 μM). After incubation for 30 min at 37°C, then monitored by excitation at 355nm and emission at 460 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (4-methylumbelliferyl sulfate potassium).

Reference:

S. Caroselli et al., Discovery of the First Human Arylsulfatase A Reversible Inhibitor Impairing Mouse Oocyte Fertilization. *ACS Chem Biol* 15, 1349-1357 (2020).

1. **Creatine kinase (EC: 2.7.3.2)**

Reaction:

ATP + creatine = ADP + H+ + N-phosphocreatine (reversible reaction)



Reaction system: Based on the protocol of the detection kit (Sigma Cat# MAK116). Secreted protein extracting solution (final concentration at 10 μg/mL) was added to reaction buffer and reaction with N-phosphocreatine and ADP to generate creatine and ATP, and ATP was further reacted with hexokinase and NADP+, which along with the formation of G6P and NADPH, which lead to the increase of absorbance at 340 nm. After incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (N-phosophocreatine).

Reference:

A. Sinadinos *et al.*, P2RX7 purinoceptor: a therapeutic target for ameliorating the symptoms of duchenne muscular dystrophy. *PLoS Med* **12**, e1001888 (2015).

1. **Prolyl oligopeptidase (EC: 3.4.21.26)**

Reaction:

Hydrolysis of Pro-|-Xaa >> Ala-|-Xaa in oligopeptides.

Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and reacted by the addition of 200 μM Z-Gly-Pro-pNA. After incubation for 30 min at 37°C, the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (Z-Gly-Pro-pNA).

Reference:

P. L. Mäkinen, K. K. Mäkinen, S. A. Syed, An endo-acting proline-specific oligopeptidase from Treponema denticola ATCC 35405: evidence of hydrolysis of human bioactive peptides. *Infect Immun* **62**, 4938-4947 (1994).

1. **Dimethylallyltranstransferase (EC 2.5.1.1)**

Reaction:

dimethylallyl diphosphate + isopentenyl diphosphate = (2E)-geranyl diphosphate + diphosphate



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to PBS buffer, containing MgCl2 (Cfinal = 5 mM) and Triton X100 (0.01%), and reacted by the addition of geranyl diphosphate (GPP; Cfinal = 100 μM), isopentenyl diphosphate (Cfinal = 100 μM). After incubation for 30 min at 37°C, the generated diphosphate was measured by phosphate detection kit. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (GPP and isopentenyl diphosphate).

Reference:

S. Lindert *et al.*, Farnesyl diphosphate synthase inhibitors from in silico screening. *Chem Biol Drug Des* **81**, 742-748 (2013).

1. **Arginase (EC: 3.5.3.1)**

Reaction:

H2O + L-arginine = L-ornithine + urea



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and reacted by the addition of 500 μM arginine. After incubation for 30 min at 37°C, the product urea was detected with α–isonitrosopropiophenone in the acid buffer and absorbance was measured at 550 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (L-arginine).

Reference:

O. Schnorr *et al.*, Cocoa flavanols lower vascular arginase activity in human endothelial cells in vitro and in erythrocytes in vivo. *Arch Biochem Biophys* **476**, 211-215 (2008).

1. **Beta-galactosidase (EC 3.2.1.23)**

Reaction:

Hydrolysis of terminal non-reducing beta-D-galactose residues in beta-D-galactosides.

Reaction system: Based on the protocol of the detection kit (Solarbio Cat# BC2580). Secreted protein extracting solution (Cfinal = 10 μg/mL) was added to reaction buffer and reaction with p-nitrobenzene-β-D-galactoside, after incubation for 30 min at 37°C, the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (p-nitrobenzene-β-D-galactoside).

Reference:

Q. H. Fan, K. A. Claunch, S. Striegler, Structure-activity relationship of highly potent galactonoamidine inhibitors toward β-galactosidase (Aspergillus oryzae). *J Med Chem* **57**, 8999-9009 (2014).

1. **Guanine deaminase (EC: 3.5.4.3)**

Reaction:

guanine + H+ + H2O = NH4+ + xanthine



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and reacted by the addition of 1 mM guanine. After incubation for 30 min at 37°C, and the absorbance was measured at 245 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (guanine).

Reference:

S. Kumar, V. Josan, K. C. Sanger, K. K. Tewari, P. S. Krishnan, Studies on guanine deaminase and its inhibitors in rat tissue. *Biochem J* 102, 691-704 (1967).

1. **Inositol-phosphate phosphatase (EC: 3.1.3.25)**

Reaction:

a myo-inositol phosphate + H2O = myo-inositol + phosphate



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer containing 5 mM β-glycerophosphate. After incubation for 30 min at 37°C, the reaction was terminated by the addition of 0.5% ammonium molybdate, 1% triton X-100, 4.5% H2SO4 in water. the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (β-glycerophosphate).

Reference:

P. Vincendon et al., An automated high volume assay to screen for inhibitors of myo-inositol monophosphatase from microbial fermentation broths. *J Antibiot* (Tokyo) 49, 710-712 (1996).

1. **Nitric-oxide synthase (NADPH) (EC 1.14.13.39)**

Reaction:

H+ + 2 L-arginine + 3 NADPH + 4 O2 = 4 H2O + 2 L-citrulline + 3 NADP+ + 2 nitric oxide



Reaction system: secreted protein extracting solution (Cfinal = 10 μg/mL) was added to ammonia-ammonium chloride buffer containing L-arginine (Cfinal = 2 mM), NADPH (Cfinal = 600 μM), after incubation for 30 min at 37°C, and the mixture was further reacted by adding DAF-FM DA (Cfinal = 2 mM), then monitored by excitation at 495 nm and emission at 515 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (L-arginine).

Reference:

S. Agrawal, R. Kumari, P. M. Luthra, A reliable fluorimetric method to screen the nitric oxide synthase inhibitors in 96 well plate. *Anal Biochem* **577**, 42-44 (2019)

1. **Dihydrofolate reductase (EC: 1.5.1.3)**

Reaction:

7,8-dihydrofolate + H+ + NADPH = (6S)-5,6,7,8-tetrahydrofolate + NADP+



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer (containing 500 μM mercaptoethanol), and reacted by the addition of 400 μM dihydrofolate, and 200 μM NAPDH. After incubation for 30 min at 37°C, and the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (dihydrofolate).

Reference:

L. N. Heppler *et al.*, The antimicrobial drug pyrimethamine inhibits STAT3 transcriptional activity by targeting the enzyme dihydrofolate reductase. *J Biol Chem* **298**, 101531 (2022).

1. **Hydroxymethylglutaryl-CoA reductase (NADPH) (EC: 1.1.1.34)**

Reaction:

(3S)-hydroxy-3-methylglutaryl-CoA + 2 H+ + 2 NADPH = (R)-mevalonate + CoA + 2 NADP+



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and reacted by the addition of 100 μM HMG-CoA, and 100 μM NADPH. After incubation for 30 min at 37°C, and the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (HMG-CoA).

Reference:

G. Baskaran *et al.*, HMG-CoA reductase inhibitory activity and phytocomponent investigation of Basella alba leaf extract as a treatment for hypercholesterolemia. *Drug Des Devel Ther* **9**, 509-517 (2015).

1. **Alkaline phosphatase (EC 3.1.3.1)**

Reaction:

a phosphate monoester + H2O = an alcohol + phosphate



Reaction system: Based on the protocol of the detection kit (Solarbio Cat# BC2140). Secreted protein extracting solution (final concentration at 10 μg/mL) was added to reaction buffer and reaction with para-nitrophenyl phosphate (pNPP), after incubation for 30 min at 37°C, the generated para-nitrophenol show yellow under alkaline condition, and the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (pNP).

Reference:

Y. Mei *et al.*, Fluorescence quenching based alkaline phosphatase activity detection. *Talanta* **176**, 52-58 (2018).

1. **Sulfite oxidase (EC: 1.8.3.1)**

Reaction:

H2O + O2 + sulfite = H2O2 + sulfate



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and reacted by the addition of 400 μM Potassium ferricyanide, and 100 μM Na2SO3. After incubation for 30 min at 37°C and the absorbance was measured at 420 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (Na2SO3).

Reference:

S. Agrawal, R. Kumari, P. M. Luthra, A reliable fluorimetric method to screen the nitric oxide synthase inhibitors in 96 well plate. *Anal Biochem* 577, 42-44 (2019).

1. **Long-chain-fatty-acid—CoA ligase (EC: 6.2.1.3)**

Reaction:

a long-chain fatty acid + ATP + CoA = a long-chain fatty acyl-CoA + AMP + diphosphate



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and reacted by the addition of 25 μM CoASH, 10 μM ATP, and 5 μM sodium oleate. After incubation for 30 min at 37°C, the remaining ATP was measured by ATP detection kit. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (sodium oleate).

Reference:

L. Turcano et al., Identification by High-Throughput Screening of Pseudomonas Acyl-Coenzyme A Synthetase Inhibitors. *SLAS Discov* 22, 897-905 (2017).

1. **Phospholipase A2 (EC: 3.1.1.4)**

Reaction:

a 1,2-diacyl-sn-glycero-3-phosphocholine + H2O = a 1-acyl-sn-glycero-3-phosphocholine + a fatty acid + H+



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and the reaction was started by the addition of 500 μM 1,2-bis(heptanoylthio) glycerophosphocholine, 500 μM DTNB in PBS buffer. After incubation for 30 min at 37°C, the absorbance was measured at 420 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (1,2-bis(heptanoylthio) glycerophosphocholine).

Reference:

H. Chen et al., Discovery of a novel pyrazole series of group X secreted phospholipase A2 inhibitor (sPLA2X) via fragment based virtual screening. *Bioorg Med Chem Lett* 24, 5251-5255 (2014).

1. **Peptidylprolyl isomerase (EC: 5.2.1.8)**

Reaction:

[protein]-peptidylproline (omega=180) = [protein]-peptidylproline (omega=0)



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer containing 1.5 mM succinyl-Ala-Phe-Pro-Phe-4-nitroanilide, and the reaction was started by the addition of 0.5 mg chymotrypsin. After incubation for 10 min at 37°C, the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (succinyl-Ala-Phe-Pro-Phe-4-nitroanilide).

Reference:

M. Vivoli et al., A miniaturized peptidyl-prolyl isomerase enzyme assay. *Anal Biochem* 536, 59-68 (2017).

1. **Beta-glucosidase (EC: 3.2.1.21)**

Reaction:

a beta-D-glucosyl-(1<->1')-N-acylsphing-4-enine + H2O = an N-acylsphing-4-enine + D-glucose



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and the reaction was started by the addition of substrate (200 μM 4-Nitrophenyl β-D-glucopyranoside). After incubation for 30 min at 37°C, the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (4-Nitrophenyl β-D-glucopyranoside).

Reference:

G. Y. Chen, H. Zhang, F. Q. Yang, A simple and portable method for β-Glucosidase activity assay and its inhibitor screening based on a personal glucose meter. *Anal Chim Acta* **1142**, 19-27 (2021).

1. **Insulysin (EC: 3.4.24.56)**

Reaction:

Degradation of insulin, glucagon and other polypeptides.

Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer (containing 10 μM ZnSO4), and reacted by the addition of 50 nM insulin. After incubation for 30 min at 37°C, the remaining insulin was measured by the insulin detection kit. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (insulin).

Reference:

F. Leroux *et al.*, Identification of ebselen as a potent inhibitor of insulin degrading enzyme by a drug repurposing screening. *Eur J Med Chem* **179**, 557-566 (2019).

1. **CTP synthase (glutamine hydrolyzing) (EC: 6.3.4.2)**

Reaction:

ATP + H2O + L-glutamine + UTP = ADP + CTP + 2 H+ + L-glutamate + phosphate



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and reacted by the addition of 600 μM UTP, 1.5 mM ATP, 0.2 mM GTP, 10 mM glutamine. After incubation for 30 min at 37°C and the absorbance was measured at 291 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (UTP, glutamine).

Reference:

E. M. Lynch et al., Human CTP synthase filament structure reveals the active enzyme conformation. *Nat Struct Mol Biol* 24, 507-514 (2017).

1. **Glutaminase (EC: 3.5.1.2)**

Reaction:

H2O + L-glutamine = L-glutamate + NH4+



Reaction system: Based on the protocol of the detection kit (Solarbio Cat# BC1450). Secreted protein extracting solution (final concentration at 10 μg/mL) was added to reaction buffer and reaction with L-glutamine, after incubation for 30 min at 37°C, the generated ammonia was reacted with Nessler Reagent, and the absorbance was measured at 420 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (L-glutamine).

Reference:

T. M. Maharem, M. A. Emam, Y. A. Said, Purification and characterization of l-glutaminase enzyme from camel liver: Enzymatic anticancer property. *Int J Biol Macromol* **150**, 1213-1222 (2020).

1. **Alpha-glucosidase (EC: 3.2.1.20)**

Reaction:

Hydrolysis of terminal, non-reducing (1->4)-linked alpha-D-glucose residues with release of alpha-D-glucose.

Reaction system: Secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and then added 200 μM 4-Nitrophenyl α-D-glucopyranoside, after incubation for 30 min at 37°C, the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (4-Nitrophenyl α-D-glucopyranoside).

Reference:

D. M. Liu, J. Chen, Y. P. Shi, α-Glucosidase immobilization on chitosan-enriched magnetic composites for enzyme inhibitors screening. *Int J Biol Macromol* **105**, 308-316 (2017).

1. **3-oxo-5alpha-steroid 4-dehydrogenase (NADP(+)) (EC: 1.3.1.22)**

Reaction:

a 3-oxo-5alpha-steroid + NADP+ = a 3-oxo-Delta4-steroid + H+ + NADPH



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and the reaction was started by the addition of 200 μM testosterone, 800 μM NADPH. After incubation for 10 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (testosterone).

Reference:

A. Nahata, V. K. Dixit, Evaluation of 5α-reductase inhibitory activity of certain herbs useful as antiandrogens. *Andrologia* **46**, 592-601 (2014).

1. **Procollagen-proline 3-dioxygenase (EC: 1.14.11.7)**

Reaction:

2-oxoglutarate + L-prolyl-[collagen] + O2 = CO2 + succinate + trans-3-hydroxy-L-prolyl-[collagen]



Reaction system: Secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer containing 0.6 mg/ml catalase, 500 μM 2-oxoglutarate, 500 μM HIF-1 alpha (556-574) and incubation for 10 min at 37°C. Then, after adding 10 mg/mL in 0.5 M HCl and incubation for 10 min at 95°C, 30 μL 1.25 M NaOH was added and then monitored by excitation at 340 nm and emission at 420 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (2-oxoglutarate, HIF-1 alpha (556-574)).

Reference:

Y. Wu, Z. Jiang, Q. You, X. Zhang, Application of in-vitro screening methods on hypoxia inducible factor prolyl hydroxylase inhibitors. *Bioorg Med Chem* **25**, 3891-3899 (2017).

1. **Glutathione transferase (EC: 2.5.1.18)**

Reaction:

glutathione + RX = a halide anion + an S-substituted glutathione + H+



Reaction system: Based on the protocol of the detection kit (Solarbio Cat# BC0350). Secreted protein extracting solution (final concentration at 10 μg/mL) was added to reaction buffer and reaction with 1-chlom-2,4-dinitrobenzene (CDNB). After incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (CDNB).

Reference:

K. P. C. Ozelame *et al.*, Novel tick glutathione transferase inhibitors as promising acaricidal compounds. *Ticks Tick Borne Dis* **13**, 101970 (2022)

1. **Protein disulfide-isomerase (EC: 5.3.4.1)**

Reaction:

Catalyzes the rearrangement of -S-S- bonds in proteins.

Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to assay buffer (containing 50mM Tris-HCl buffer, 1 mM EDTA, 2 mM DTT), and the reaction was started by the addition of 75 μM insulin. After incubation for 30 min at 37°C, the absorbance was measured at 630 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (insulin).

Reference:

G. Liu *et al.*, Improvements of Modified Wheat Protein Disulfide Isomerases with Chaperone Activity Only on the Processing Quality of Flour. *Food and Bioprocess Technology* **10**, 568-581 (2017).

1. **Acetyl-CoA carboxylase (EC: 6.4.1.2)**

Reaction:

acetyl-CoA + ATP + hydrogencarbonate = ADP + H+ + malonyl-CoA + phosphate



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to assay buffer (50 mM Tris-acetate, 16 mM NaHCO3, 0.9 mg/mL BSA, β-mercaptoethanol, 4.3 mM magnesium acetate), and the reaction was started by the addition of 25 μM ATP, 0.25 mM acetyl CoA. After incubation for 30 min at 37°C, the remaining ATP was detected by ATP detection kit. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (acetyl CoA).

Reference:

S. Keil et al., Identification and synthesis of novel inhibitors of acetyl-CoA carboxylase with in vitro and in vivo efficacy on fat oxidation. *J Med Chem* 53, 8679-8687 (2010).

1. **Peptidyl-dipeptidase A (EC: 3.4.15.1)**

Reaction:

angiotensin I + H2O = angiotensin II + L-histidyl-L-leucine



Reaction system: Secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and then added 200 μM N-[3-(2-Furyl)acryloyl]-Phe-Gly-Gly, after incubation for 30 min at 37°C, the absorbance was measured at 405 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (N-[3-(2-Furyl)acryloyl]-Phe-Gly-Gly).

Reference:

M. Y. Ali, S. Zaib, S. Jannat, I. Khan, Inhibition of Angiotensin-I Converting Enzyme by Ginsenosides: Structure-Activity Relationships and Inhibitory Mechanism. *J Agric Food Chem* **69**, 6073-6086 (2021).

1. **Histidine decarboxylase (EC: 4.1.1.22)**

Reaction:

H+ + L-histidine = CO2 + histamine



Reaction system: Secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and then added 60 μM histidine hydrochloride, 100 μM reduced glutathione, and 100 μM histidine, after incubation for 30 min at 37°C, the product histamine was measured by histamine Elisa kit (Cat# SEKSM-0029). For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (histidine).

Reference:

H. Hanieh et al., Pinocembrin, a novel histidine decarboxylase inhibitor with anti-allergic potential in in vitro. *Eur J Pharmacol* 814, 178-186 (2017).

1. **Carbamoyl-phosphate synthase (ammonia) (EC: 6.3.4.16)**

Reaction:

2 ATP + hydrogencarbonate + NH4+ = 2 ADP + carbamoyl phosphate + 2 H+ + phosphate



Reaction system: the enzymatic activity of CPS was determined by coupling carbamoyl phosphate formation with aspartate carbamoyl transferase (ATCase). Secreted protein extracting solution (final concentration at 10 μg/mL) was added to assay buffer (20mM Tris-HCl (pH 8.0), 100mM KCl, 5mM NaHCO3, 10mM MgCl2, 10mM ATP), and the reaction was started by the addition of 5mM Glutamine, 5mM UMP, 5mM L-aspartate, and 0.1 g/L ATCase. After incubation for 30 min at 37°C, the absorbance was measured at 466 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (hydrogencarbonate, NH4+).

Reference:

S. Shen, X. Zhang, Z. Li, Development of an engineered carbamoyl phosphate synthetase with released sensitivity to feedback inhibition by site-directed mutation and casting error-prone PCR. *Enzyme Microb Technol* 129, 109354 (2019).

1. **Thymidylate synthase (EC: 2.1.1.45)**

Reaction:

(6R)-5,10-methylene-5,6,7,8-tetrahydrofolate + dUMP = 7,8-dihydrofolate + dTMP



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer (containing 20 mM MgCl2), and reacted by the addition of 1mM dUMP and 30 mM (6R,S)-methyltetrahydrofolate. After incubation for 30 min at 37°C, and the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (dUMP).

Reference:

Z. M. M. Alzhrani, M. M. Alam, T. Neamatallah, S. Nazreen, Design, synthesis and in vitro antiproliferative activity of new thiazolidinedione-1,3,4-oxadiazole hybrids as thymidylate synthase inhibitors. *J Enzyme Inhib Med Chem* 35, 1116-1123 (2020).

1. **Malate dehydrogenase (EC: 1.1.1.37)**

Reaction:

H+ + NADH + oxaloacetate = (S)-malate + NAD+ (reversible reaction )



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and the reaction was started by the addition of 200 μM oxaloacetic acid, and 200 μM NADH. After incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (oxaloacetate).

Reference:

H. S. Ban et al., A Novel Malate Dehydrogenase 2 Inhibitor Suppresses Hypoxia-Inducible Factor-1 by Regulating Mitochondrial Respiration. *PLoS One* 11, e0162568 (2016).

1. **Fructose-bisphosphatase (EC: 3.1.3.11)**

Reaction:

beta-D-fructose 1,6-bisphosphate + H2O = beta-D-fructose 6-phosphate + phosphate



Reaction system: Based on the protocol of the detection kit (Mreda Cat# M054997). Briefly, secreted protein extracting solution (final concentration at 10 μg/mL) was reacting with β-D-fructose 1, 6-bisphosphate, after incubation for 30 min at 37°C, the generated β-D-fructose 6-phosphate was coupling with phosphoglucose isomerase and glucose 6-phosphate dehydrogenase to generate phosphogluconic acid and NADP+, the absorbance change was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (β-D-fructose 1, 6-bisphosphate).

Reference:

B. R. Liao *et al.*, Synthesis and structure-activity relationship of non-phosphorus-based fructose-1,6-bisphosphatase inhibitors: 2,5-Diphenyl-1,3,4-oxadiazoles. *Eur J Med Chem* **83**, 15-25 (2014).

1. **Chitinase (EC: 3.2.1.14)**

Reaction:

Random endo-hydrolysis of N-acetyl-beta-D-glucosaminide (1->4)-beta-linkages in chitin and chitodextrins.

Reaction system: Secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and then added 22 μM 4-methylumbelliferyl-D-N-N'-N''-triacetylchitotriose, after incubation for 30 min at 37°C, the absorbance was monitored by excitation at 366 nm and emission at 445 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: the mimics of natural substrate (4-methylumbelliferyl-D-N-N'-N''-triacetylchitotriose).

Reference:

C. E. Hollak, S. van Weely, M. H. van Oers, J. M. Aerts, Marked elevation of plasma chitotriosidase activity. A novel hallmark of Gaucher disease. *J Clin Invest* 93, 1288-1292 (1994).

1. **Lactoylglutathione lyase (EC: 4.4.1.5)**

Reaction:

glutathione + methylglyoxal = (R)-S-lactoylglutathione (reversible reaction)



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and reacted by the addition of 300 μM Methylglyoxal, and 300 μM L-Glutathione. After incubation for 10 min at 25°C, the absorbance was measured at 240 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (Methylglyoxal, and L-Glutathione).\

Reference:

S. Chakraborty, M. Gogoi, D. Chakravortty, Lactoylglutathione lyase, a critical enzyme in methylglyoxal detoxification, contributes to survival of Salmonella in the nutrient rich environment. *Virulence* **6**, 50-65 (2015).

1. **3beta-hydroxy-Delta(5)-steroid dehydrogenase (****EC: 1.1.1.145)**

Reaction:

a 3beta-hydroxy-Delta5-steroid + NAD+ = a 3-oxo-Delta5-steroid + H+ + NADH



Reaction system: Secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and then added 1 mM DHEA, 200 μM NAD+, after incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (DHEA).

Reference:

J. L. Thomas, K. M. Bucholtz, B. Kacsoh, Selective inhibition of human 3β-hydroxysteroid dehydrogenase type 1 as a potential treatment for breast cancer. *J Steroid Biochem Mol Biol* 125, 57-65 (2011).

1. **Dihydropyrimidine dehydrogenase (NADP(+)) (EC: 1.3.1.2)**

Reaction:

H+ + NADPH + uracil = 5,6-dihydrouracil + NADP+



Reaction system: secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer (20 μM dithioerythritol), and the reaction was started by the addition of 600 μM NADPH, 1.5 mM uracil. After incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (uracil).

Reference:

Y. Camadan, H. Özdemir, İ. Gulcin, Purification and characterization of dihydropyrimidine dehydrogenase enzyme from sheep liver and determination of the effects of some anaesthetic and antidepressant drugs on the enzyme activity. *J Enzyme Inhib Med Chem* **31**, 1335-1341 (2016).

1. **Tyrosinase (EC: 1.14.18.1)**

Reaction:

2 L-dopa + O2 = 2 H2O + 2 L-dopaquinone



Reaction system: Secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and then added 1 mM L-dopa, after incubation for 30 min at 37°C, the absorbance was measured at 475 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (L-dopa).

Reference:

Y. He, T. L. Suyama, H. Kim, E. Glukhov, W. H. Gerwick, Discovery of Novel Tyrosinase Inhibitors from Marine Cyanobacteria. *Front Microbiol* **13**, 912621 (2022).

1. **Methylenetetrahydrofolate reductase [NAD(P)H] (EC:** **1.5.1.20)**

Reaction:

(6S)-5-methyl-5,6,7,8-tetrahydrofolate + NADP+ = (6R)-5,10-methylene-5,6,7,8-tetrahydrofolate + H+ + NADPH



Reaction system: Secreted protein extracting solution (final concentration at 10 μg/mL) was added to 50 mM phosphate buffer containing 10% glycerol and 0.3 mM EDTA, and then added 1.4 mM (6S)-S-methyl-5,6,7,8-tetrahydrofolate, 400 μM NADH, after incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate ((6S)-S-methyl-5,6,7,8-tetrahydrofolate).

Reference:

M. Grabowski et al., Genistein inhibits activities of methylenetetrahydrofolate reductase and lactate dehydrogenase, enzymes which use NADH as a substrate. *Biochem Biophys Res Commun* 465, 363-367 (2015).

1. **Glutamate dehydrogenase [NAD(P)(+)] (EC: 1.4.1.3)**

Reaction:

H2O + L-glutamate + NAD+ = 2-oxoglutarate + H+ + NADH + NH4+



Reaction system: Secreted protein extracting solution (final concentration at 10 μg/mL) was added to PBS buffer, and then added 10 mM L-Glutamate, 200 μM NAD+, after incubation for 30 min at 37°C, the absorbance was measured at 340 nm. For blank control, unfermented BHI medium extracting solution was used to replace protein extracting solution.

Substrate: natural substrate (L-Glutamate).

Reference:

M. De Angelis *et al.*, NADP-glutamate dehydrogenase activity in nonstarter lactic acid bacteria: effects of temperature, pH and NaCl on enzyme activity and expression. *J Appl Microbiol* **109**, 1763-1774 (2010).