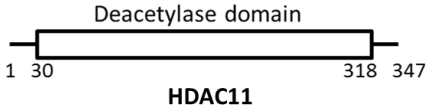
HDAC11 protein purification

Human HDAC11, a 347 amino acid long protein, was cloned in 2002. It is the only member of Class IV HDACs and consists of only a deacetylase domain. It shows tissue-specific expression, with high expression in heart, kidney, brain, testis and skeletal muscle (Gao et al., 2002). In addition to exhibiting its repressive effect, it has also been involved in activation of genes when present in complex with another HDAC. HDAC11 has been shown to inhibit IL-10 gene expression, but when present with HDAC6, IL-10 expression is activated (Cheng et al., 2014; Villagra et al., 2009). Recent studies have pointed out its expression and role in the hematopoietic cells, for example, T-cells, antigen presenting cells, neutrophils and myeloid derived suppressor cells (Huang et al., 2017; Sahakian et al., 2017; Sahakian et al., 2015; Villagra et al., 2009; Woods et al., 2017). It has been found to be implicated in colon, prostrate, ovarian and breast cancers, pituitary tumors, pancreatic neuroendocrine tumor, gliomas and renal I/R injury (Dali-Youcef et al., 2015; Klieser et al., 2017; Mrug and Sanders, 2013; Wang et al., 2017; Zhang et al., 2017). In a recent study, its inhibition has shown to enhance the radiation lethality in cell lines (Kim et al., 2016).

In order to develop a high-throughput assay for HDAC11, a good quality preparation with the best possible yield of HDAC11 protein is the starting point. Following are the details for HDAC11 purification.

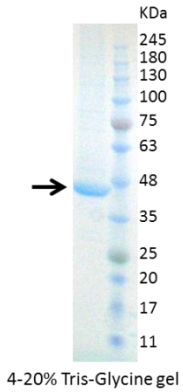


Construct length: Full length (1-347 amino acids)

Vector: pFBOH-LIC

Expression cells: *Sf9*

Tag: None (His-tag cut with thrombin protease) Conc.: 1.4 mg/ml Yield: 6 mg/L



The harvested cells were re-suspended in 20 mM Tris-HCl buffer, pH 7.5, containing 500 mM NaCl, 5 mM imidazole, 5% glycerol and 1X complete EDTA-free protease inhibitor cocktail tablet (Roche). The cells were lysed chemically by rotating for 30 min with NP40 (final concentration of 0.6%), 22.5 U/mL Benzonase nuclease (Sigma) and 1 mM TCEP followed by sonication at frequency of 7.5 (10” on/10” off)

for 2 min (Sonicator 3000, Misoni). The crude extract was clarified by high-speed centrifugation (60 min at 36,000 ×g at 4˚C) by Beckman Coulter centrifuge.

The supernatent was then incubated with Ni-NTA resin pre-equilibrated with 50 mM Tris (pH 8), 10% glycerol, 500 mM NaCl, 5 mM Imidazole for 2 hours. The resin was washed on the column by running 50 mM Tris-HCl, pH 8, 500 mM NaCl, 5% glycerol, containing 30 mM imidazole. The His tag was removed on the column by overnight digestion using 100 units of Thrombin (sigma) in buffer containing 50 mM Tris (pH 8), 20% glycerol, 250 mM NaCl and 2 mM TCEP (Sigma). The flow through containing cut HDAC11 was collected and the purity was confirmed on SDS-PAGE gel. The protein was concentrated (precipitated above 1.4 mg/ml) and flash frozen. The entire harvesting, cell lysis and purification was performed at 4 °C.

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