Antibody Characterization Report for Tubulin alpha-4A chain

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Tubulin alpha-4A chain

Alternative protein name: Alpha-tubulin 1, Testis-specific alpha-tubulin, Tubulin H2-alpha, Tubulin alpha-1 chain

Gene name: *TUBA4A*

Uniprot: P68366

We are a third-party organization with the mission to characterize commercial antibodies for all human protein through open science [1]. This report guides researchers to select the most appropriate antibodies for Tubulin alpha-4A chain. We used an antibody characterization pipeline based on knockout (KO) [2] to perform head-to-head comparisons of commercial antibodies for Tubulin alpha-4A chain by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HeLa and HAP1 were selected based on evidence of appropriate Tubulin alpha-4A chain expression determined through public proteomics databases, namely PaxDB [3] and DepMap [4, 5]. HeLa was modified with CRISPR/Cas9 [6] to knockout the corresponding *TUBA4A* gene. An HAP1 *TUBA4A* KO line is available at Horizon discovery and was also used in this study.

The authors do not provide an assessment of the quality of the tested antibodies as their respective performances are limited to our finite experimental conditions. The readers should interpret the present findings based on their own scientific expertise. The authors acknowledge that an antibody that demonstrates specificity in the stated test conditions can be suboptimal in a different experimental format or in cell lines that differ from those directly tested here.

Table 1: Summary of the Tubulin alpha-4A chain antibodies used

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Abcam	ab176560	GR177622-65	AB_2860019	recombinant-mono	EPR13478(B)	rabbit	0.17	Wb, IF
Abcam	ab177479	GR145295-3	AB_2885101	recombinant-mono	EPR13477(B)	rabbit	0.15	Wb, IP, IF
Thermo	MA5-32738	WA3186744	AB_2810015	recombinant-mono	JM73-24	rabbit	1.00	Wb, IF
GeneTex	GTX113098	40100	AB_1952430	polyclonal	-	rabbit	0.92	Wb, IF
GeneTex	GTX112653	40163	AB_10617564	polyclonal	-	rabbit	0.44	Wb, IF
Aviva Systems Biology	ARP40179	QC9356-191027	AB_2048617	polyclonal	-	rabbit	0.50	Wb, IP

Wb=Western blot, IP= immunoprecipitation, IF=immunofluorescence

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
ATCC	CCL-2	CVCL_0030	HeLa	WT
Montreal Neurological Institute	-	CVCL_B4H8	HeLa	*TUBA4A* KO
Horizon Discovery	C631	CVCL_Y019	HAP1	WT
Horizon Discovery	HZGHC003761c001	CVCL_TV14	HAP1	*TUBA4A* KO

Figure 1: Tubulin alpha-4A chain antibody screening by immunoblot.

Lysates of HeLa and HAP1 (WT and *TUBA4A* KO) were prepared and 50 µg of protein were processed for immunoblot with the indicated Tubulin alpha-4A chain antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: ab52866 at 1/3000; ab176560 at 1/1000; ab177479 at 1/1000; MA5-32738 at 1/1000; GTX113098 at 1/1000; GTX112653 at 1/1000; ARP40179 at 1/500. Predicted band size: 50 kDa.

Figure 2: Tubulin alpha-4A chain antibody screening by immunoprecipitation.

HAP1 lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated Tubulin alpha-4A chain antibodies pre-coupled to either protein G or protein A magnetic beads. Samples were washed and processed for immunoblot with the indicated Tubulin alpha-4A chain antibody. For immunoblot, MA5-32738 was used at 1/5000. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitated.

Figure 3: Tubulin alpha-4A chain antibody screening by immunofluorescence.

A) HAP1 WT and *TUBA4A* KO cells were labelled with a green or a far-red fluorescent dye, respectively. WT and KO cells were mixed and plated to a 1:1 ratio on coverslips and fixed using paraformaldehyde (PFA). Cells were stained with the indicated Tubulin alpha-4A chain antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody including DAPI. Acquisition of the blue (nucleus-DAPI), green (identification of WT cells), red (antibody staining) and far-red (identification of KO cells) channels was performed. Representative images of the merged blue and red (grayscale) channels are shown. WT and KO cells are outlined with yellow and magenta dashed line, respectively. Antibody dilution used: ab52866 at 1/650; ab176560 at 1/200; ab177479 at 1/150; MA5-32738 at 1/1000; GTX113098 at 1/900; GTX112653 at 1/400; ARP40179 at 1/500. Bars = 10 µm.

B) WT and KO cells were identified and outlined by thresholding the green and far-red fluorescence dyes, respectively, using the Zen 3.4 (Zeiss) software. Evaluation of antibody performance was calculated by dividing the antibody mean fluorescence intensity measured from WT cells [F(WT)] by the antibody mean fluorescence intensity measured from KO cells [F(KO)]. The ratio of [F(WT)]/[F(KO)] for all tested antibodies is presented as a histogram. An antibody with a calculated ratio above 2.5-fold (dashed red line) could be considered as specific and selective for immunofluorescence. A minimum of 20 WT and 20 KO cells from 3 different fields of view were analysed for each antibody.

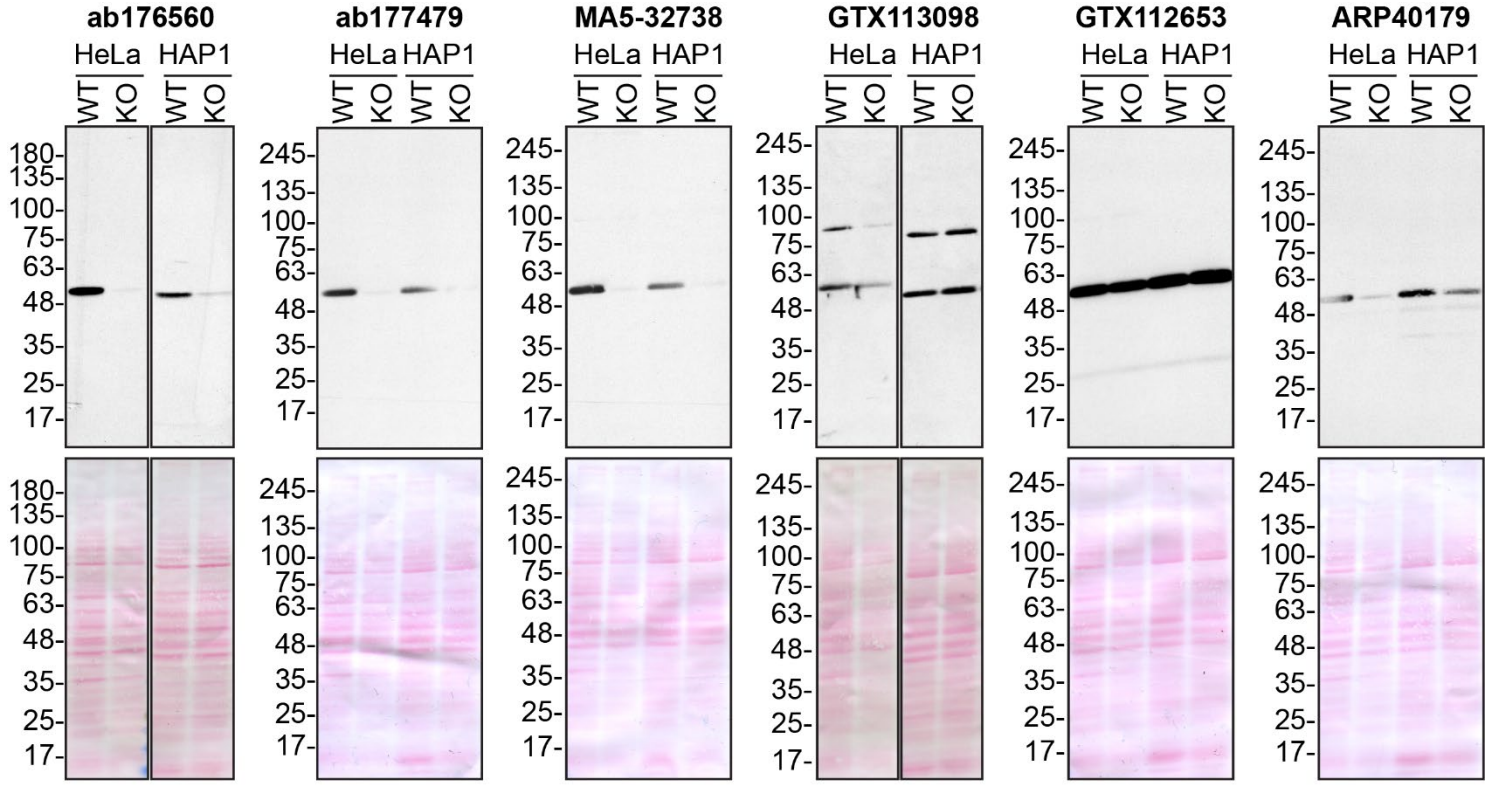

Figure 1: Tubulin alpha-4A chain antibody screening by immunoblot

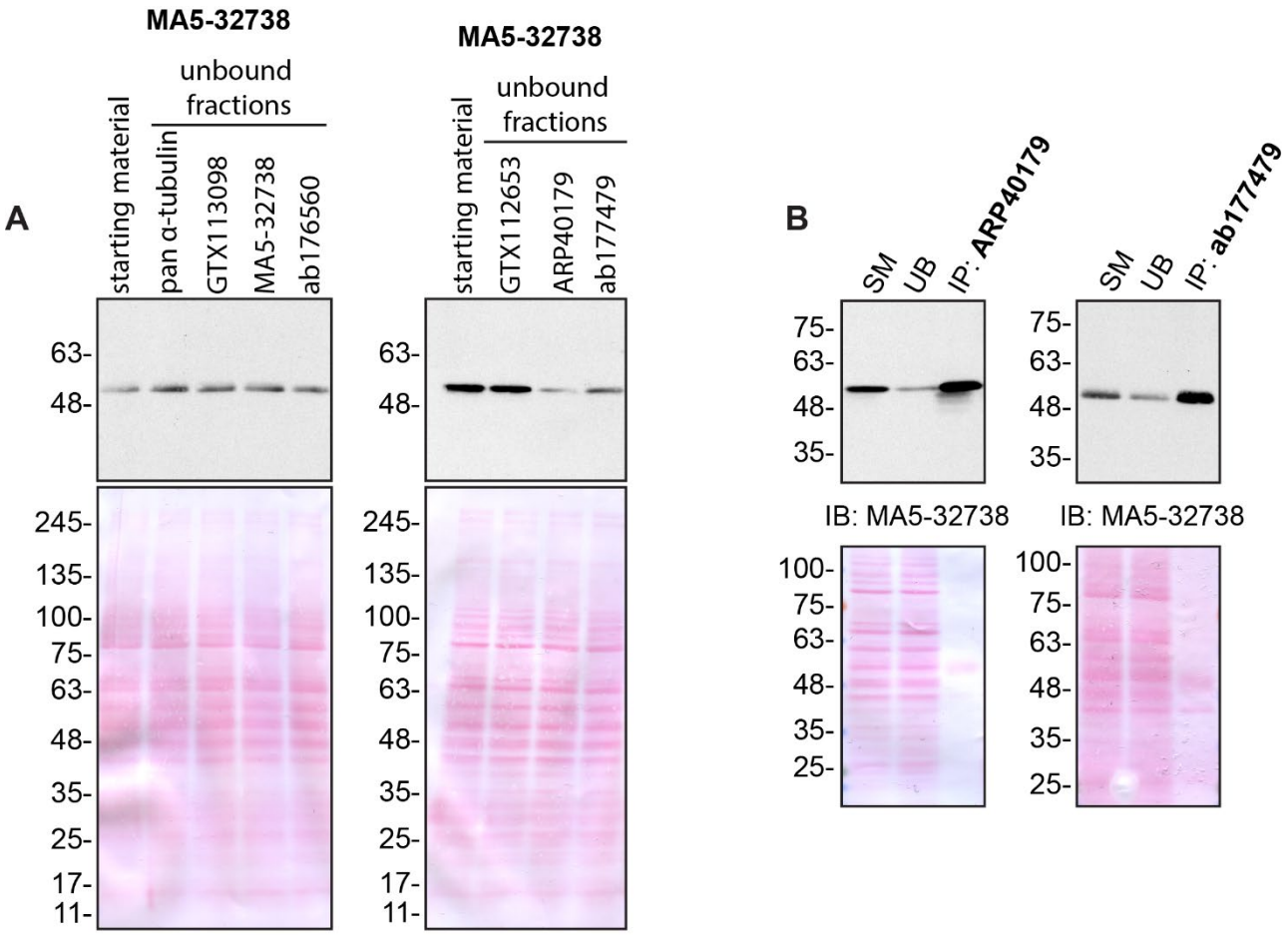

Figure 2: Tubulin alpha-4A chain antibody screening by immunoprecipitation

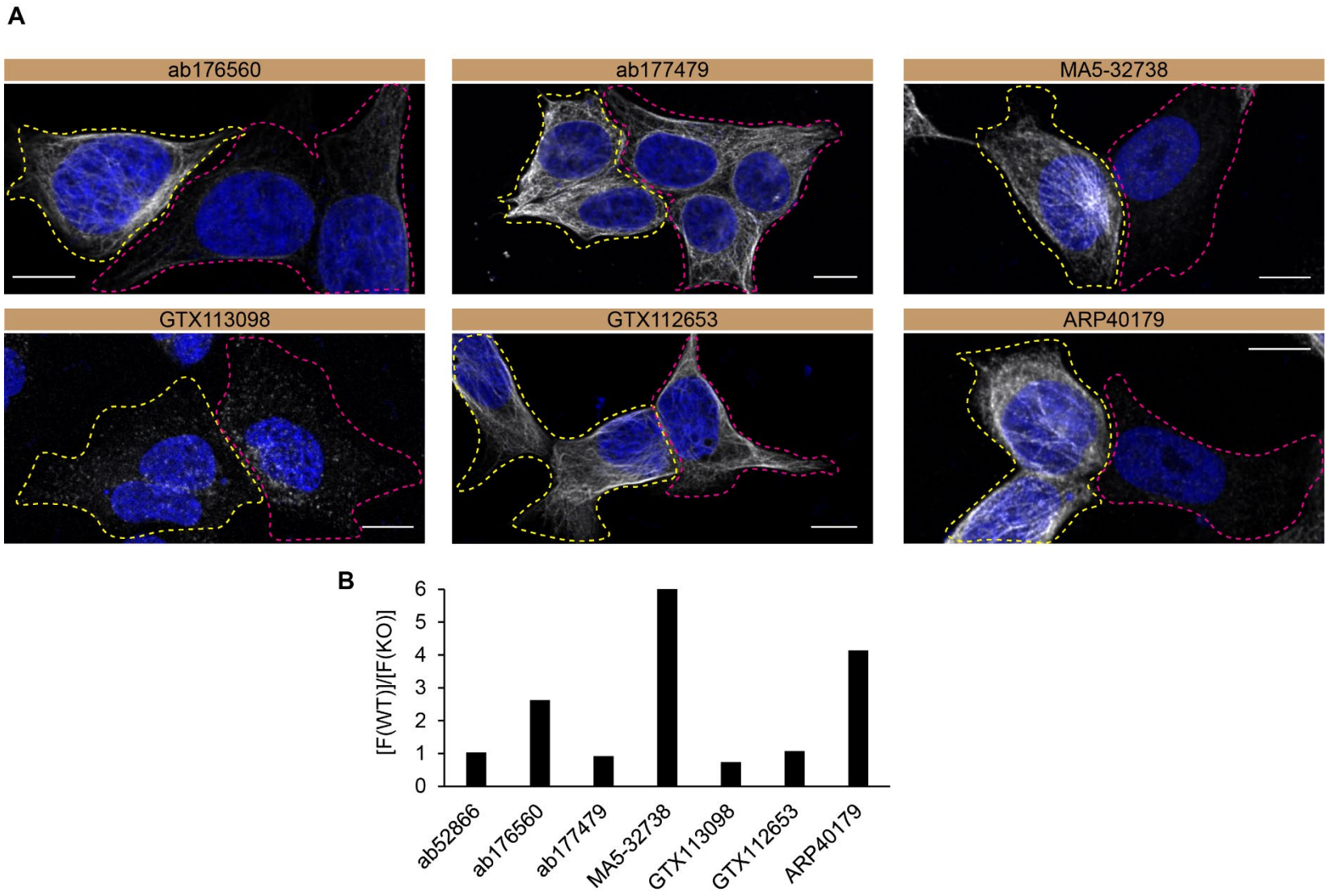

Figure 3 : Tubulin alpha-4A chain antibody screening by immunofluorescence

Materials and methods

Antibodies

All Tubulin alpha-4A chain antibodies are listed in Table 1. Peroxidase-conjugated goat anti-mouse and anti-rabbit antibodies are from Thermo Fisher Scientific (cat. number 65-6120 and 62-6520). Alexa-555-conjugated goat anti-mouse and anti-rabbit secondary antibodies are from Thermo Fisher Scientific (cat. number A21424 and A21429).

CRISPR/Cas9 genome editing

Cell lines used are listed in Table 2. HeLa *TUBA4A* KO clone was generated with low passage cells using an open-access protocol available on Zenodo.org [6]. The guide RNA sequence is CUGCUGGGAGCUCUAUUGCU.

Cell culture

Cells were cultured in DMEM high glucose (GE Healthcare cat. number SH30081.01) containing 10% fetal bovine serum (Wisent, cat. number 080450), 2 mM L-glutamate (Wisent cat. number 609065, 100 IU penicillin and 100 µg/ml streptomycin (Wisent cat. number 450201).

Antibody screening by immunoblot

Immunoblots were performed as described in our standard operating procedure [7]. HAP1 (WT and *TUBA4A* KO) were collected in RIPA buffer (25mM Tris-HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitor. Lysates were sonicated briefly and incubated 30 min on ice. Lysates were spun at ~110,000xg for 15 min at 4°C and equal protein aliquots of the supernatants were analyzed by SDS-PAGE and immunoblot. BLUelf prestained protein ladder from GeneDireX (cat. number PM008-0500) was used.

Immunoblots were performed with large 5-16% gradient polyacrylamide gels and transferred on nitrocellulose membranes. Proteins on the blots were visualized with Ponceau staining which is scanned to show together with individual immunoblot. Blots were blocked with 5% milk for 1 hr, and antibodies were incubated O/N at 4°C with 5% bovine serum albumin in TBS with 0,1% Tween 20 (TBST). Following three washes with TBST, the peroxidase conjugated secondary antibody was incubated at a dilution of ~0.2 µg/ml in TBST with 5% milk for 1 hr at room temperature followed by three washes with TBST. Membranes are incubated with ECL from

Pierce (cat. number 32106) prior to detection with HyBlot CL autoradiography films from Denville (cat. number 1159T41).

Antibody screening by immunoprecipitation

Immunoprecipitation was performed as described in our standard operating procedure [8]. Antibody-bead conjugates were prepared by adding 2.0 µg to 500 ul of Pierce IP Lysis Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together with 30µl of protein A Dynabeads from Thermo Fisher Scientific (cat. number 10002D). Pierce IP Lysis Buffer was supplemented with the Halt Protease Inhibitor Cocktail 100X from Thermo Fisher Scientific (cat. number 78446) at a final concentration of 1x. Tubes were rocked for ~2 hrs at 4°C followed by several washes to remove unbound antibodies.

HAP1 WT were collected in Pierce IP buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol) supplemented with protease inhibitor. Lysates are rocked 30 min at 4°C and spun at 110,000xg for 15 min at 4°C. One ml aliquots at 0.5 mg/ml of lysate were incubated with an antibody-bead conjugate for ~2 hrs at 4°C. Following centrifugation, the unbound fractions were collected, and beads were subsequently washed three times with 1.0 ml of IP buffer and processed for SDS-PAGE and immunoblot on 5-16% polyacrylamide gels. Prot-A:HRP (MilliporeSigma, cat. number P8651) was used as a secondary detection system at a dilution of 0.4 µg/ml for an experiment where a rabbit antibody was used for both immunoprecipitation and its corresponding immunoblot.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [9]. HAP1 WT and *TUBA4A* KO were labelled with a green and a far-red fluorescence dye, respectively. The fluorescent dyes used are from Thermo Fisher Scientific (cat. number C2925 and C34565). WT and KO cells were plated on glass coverslips as a mosaic and incubated for 24 hrs in a cell culture incubator. Cells were fixed in 4% PFA (in PBS) for 15 min at room temperature and then washed 3 times with PBS. Cells were permeabilized in PBS with 0.1% Triton X-100 for 10 min at room temperature and blocked with PBS with 5% BSA, 5% goat serum and 0.01% Triton X-100 for 30 min at room temperature. Cells were incubated with IF buffer (PBS, 5% BSA, 0.01% Triton X-100) containing the primary Tubulin alpha-4A chain antibodies O/N at 4°C. Cells were then washed 3 × 10 min with IF buffer and incubated with corresponding Alexa Fluor 555-conjugated secondary antibodies in IF buffer at a dilution of 1.0 µg/ml for 1 hr at room temperature with DAPI. Cells were washed 3 × 10 min with IF buffer and

once with PBS. Coverslips were mounted on a microscopic slide using fluorescence mounting media (DAKO).

Imaging was performed using a Zeiss LSM 700 laser scanning confocal microscope equipped with a Plan-Apo 63x oil objective (NA = 1.40). Analysis was done using the Zen navigation software (Zeiss). All cell images represent a single focal plane. Figures were assembled with Adobe Illustrator.

References

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