# Antibody Characterization Report for Transmembrane protein 106B

**YCharOS Antibody Characterization Report**

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**<u>Target</u>:**

**Recommended protein name:** Transmembrane protein 106B

**Gene name:** *TMEM106B*

**Uniprot:** Q9NUM4

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 Transmembrane protein 106B. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Transmembrane protein 106B by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HAP1 was selected based on evidence of appropriate Transmembrane protein 106B expression determined by searching DepMap [3]. An HAP1 *TMEM106B* KO line is available at Horizon discovery and was 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 Transmembrane protein 106B antibodies tested**

| Company | Catalog number | Lot number | RRID (Antibody Registry) | Clonality | Clone ID | Host | Concentration (µg/µl) | Vendors recommended applications |
|---|---|---|---|---|---|---|---|---|
| Abcam | ab244516 | GR3421643 | AB_2924268 | polyclonal | - | rabbit | 0.10 | IF |
| ABclonal | A20165 | 131370201 | AB_2862952 | polyclonal | - | rabbit | 1.88 | Wb |
| Cell Signaling Technology | 93334** | 1 | AB_2924267 | recombinant-mono | E7H7Z | rabbit | 0.10 | Wb,IP,IF |
| Proteintech | 60333-1-lg* | 10002132 | AB_2881442 | monoclonal | 5D1F8 | mouse | 2.00 | Wb |
| Thermo Fisher Scientific | PA5-34353 | XD3572518 | AB_2551705 | polyclonal | - | rabbit | 1.00 | Wb |
| Thermo Fisher Scientific | PA5-63558 | XD3571635 | AB_2648556 | polyclonal | - | rabbit | 0.10 | IF |

Wb=Western blot, IP= immunoprecipitation, IF=immunofluorescence, *=monoclonal antibody, **=recombinant antibody

**Table 2: Summary of the cell lines used**

| Institution | Catalog number | RRID (Cellosaurus) | Cell line | genotype |
|---|---|---|---|---|
| Horizon Discovery | C631 | CVCL_Y019 | HAP1 | WT |
| Horizon Discovery | HZGHC005877c010 | CVCL_XU38 | HAP1 | *TMEM106B* KO |

**Figure 1: Transmembrane protein 106B antibody screening by immunoblot.**

Lysates of HAP1 WT and *TMEM106B* KO were prepared, and 15 µg of protein were processed for immunoblot with the indicated Transmembrane protein 106B antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: ab244516 at 1/500, A20165 at 1/2000, 93334** at 1/500, 60333-1-Ig* at 1/2000, PA5-34353 at 1/500, and PA5-63558 at 1/200. Predicted band size: 31 kDa. *=monoclonal antibody, **=recombinant antibody

**Figure 2: Transmembrane protein 106B antibody screening by immunoprecipitation.**

HAP1 lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated Transmembrane protein 106B antibodies pre-coupled to Dynabeads protein G or protein A. Samples were washed and processed for immunoblot with the indicated Transmembrane protein 106B antibody. For immunoblot, 93334** was used at 1/500. The Ponceau stained transfers of each blot are shown. SM=2% starting material; UB=2% unbound fraction; IP=immunoprecipitate; HC=antibody heavy chain. *=monoclonal antibody, **=recombinant antibody.

**Figure 3: Protein antibody screening by immunofluorescence.**

HAP1 WT and *Gene* 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 in a 96-well plate with glass bottom. Cells were stained with the indicated Protein 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 blue and red (grayscale) channels are shown. WT and KO cells are outlined with green and magenta dashed line, respectively. Antibody dilution used: ab244516 at 1/100, A20165 at 1/2000, 93334** at 1/100, 60333-1-Ig* at 1/2000, PA5-34353 at 1/1000, and PA5-63558 at 1/100. Bars = 10 µm. *=monoclonal antibody, **=recombinant antibody

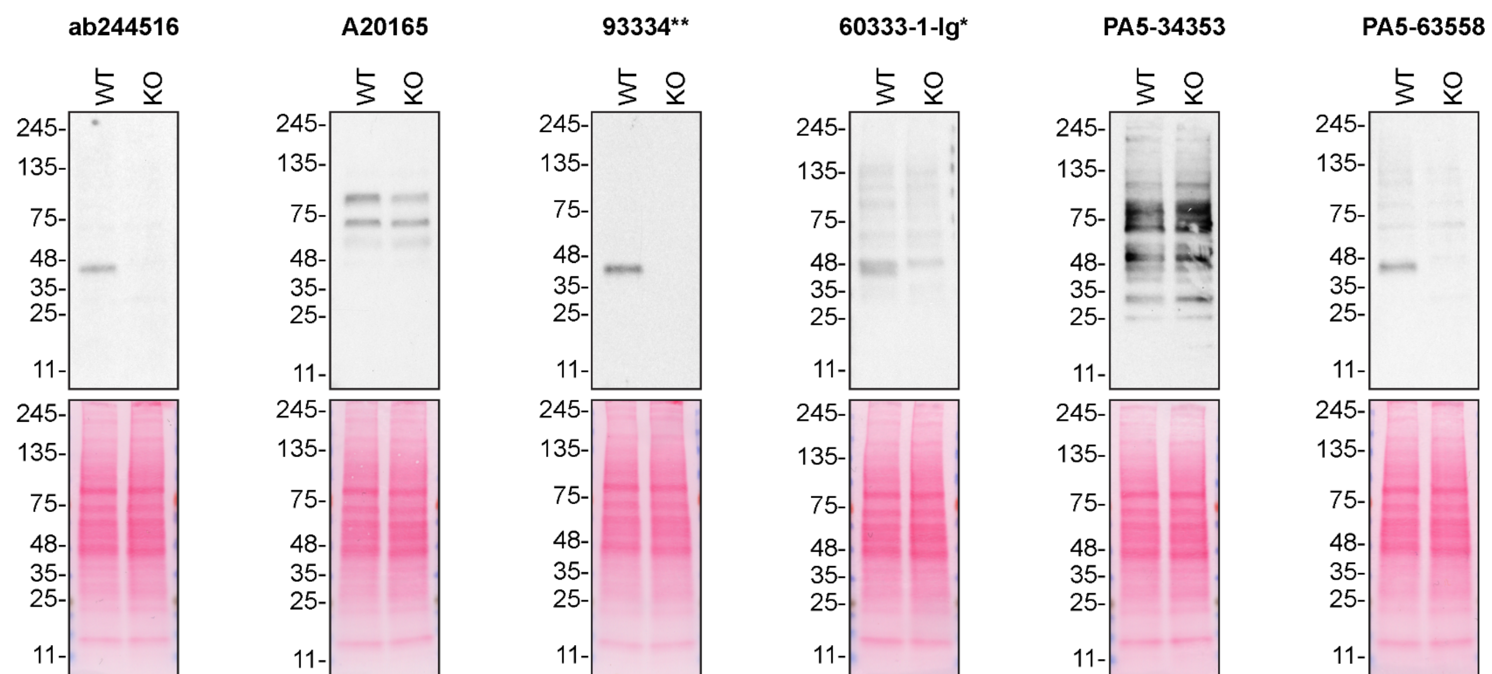


Figure 1: Transmembrane protein 106B antibody screening by immunoblot

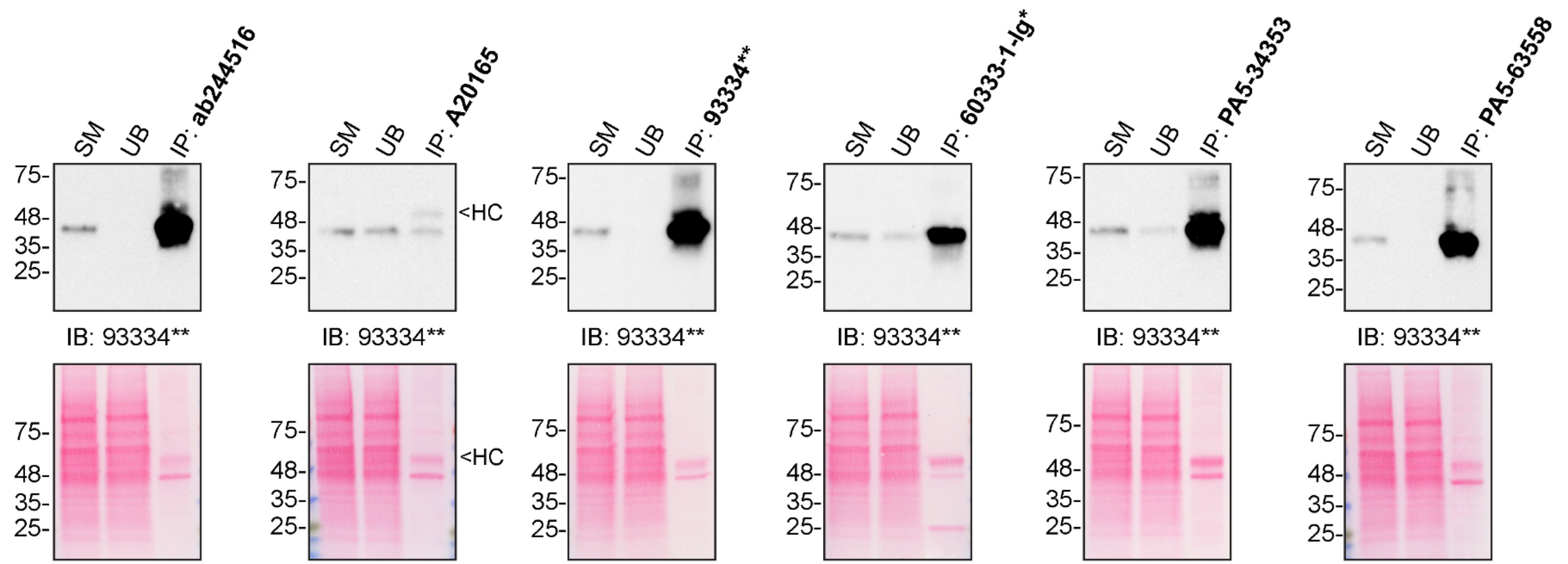


**Figure 2: Transmembrane protein 106B antibody screening by immunoprecipitation**

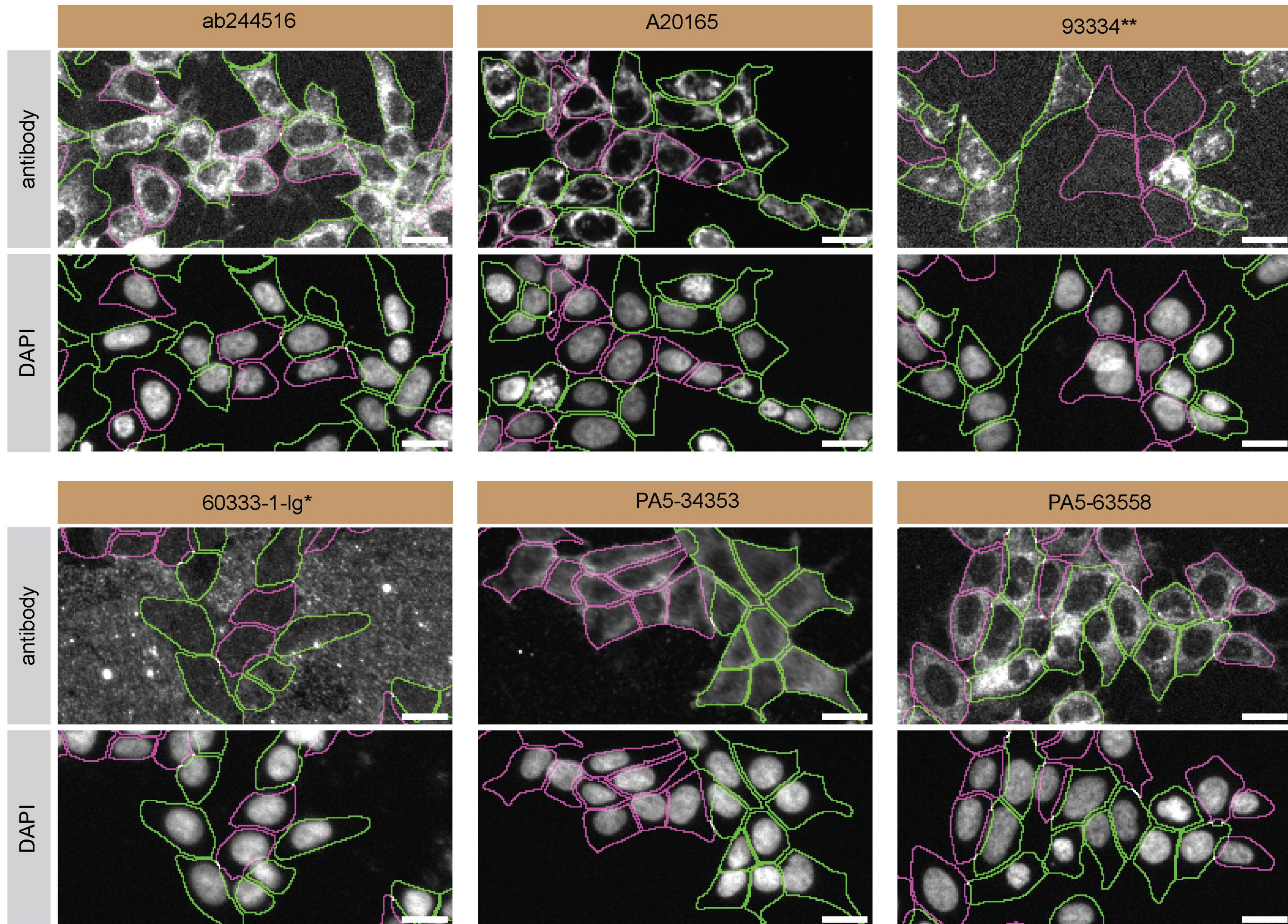


**Figure 3: Transmembrane protein 106B antibody screening by immunofluorescence**

## Materials and methods

### Antibodies

All Transmembrane protein 106B antibodies are listed in Table 1. Peroxidase-conjugated goat anti-mouse and anti-rabbit antibodies are from Thermo Fisher Scientific (cat. number 62-6520 and 65-6120).

### 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 [4]. HAP1 WT and *TMEM106B* 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 1x protease inhibitor cocktail mix (MilliporeSigma, cat. number 78429). 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 pre-cast mini 4-15% gradient polyacrylamide gels from Bio-Rad (cat. number 4561084) 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 [5]. Antibody-bead conjugates were prepared by adding 2 µg to 500 ul of Pierce IP Lysis Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together with with 30µl

of Dynabeads protein A- (for rabbit antibodies) or protein G- (for mouse antibodies) from Thermo Fisher Scientific (cat. number 10002D and 10004D, respectively). 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. 0.5 ml aliquots at 2.0 mg/ml of lysate were incubated with an antibody-bead conjugate for ~2 hrs at 4°C. The unbound fractions were collected, and beads were subsequently washed three times with 1.0 ml of IP lysis buffer and processed for SDS-PAGE and immunoblot on pre-cast mini 4-15% gradient polyacrylamide gels. Prot-A:HRP (MilliporeSigma, cat. number P8651) was used as a secondary detection system at a dilution of 0.4 µg/ml.

**Antibody screening by immunofluorescence**

Immunofluorescence was performed as described in our standard operating procedure [6]. HAP1 WT and *Gene* 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 in 96 well glass plates (Perkin Elmer, cat. number 6055300) 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 Protein 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.

Images were acquired on an ImageXpress micro widefield high-content microscopy system (Molecular Devices), using a 20x NA 0.45 air immersion objective and scientific CMOS camera, equipped with 395, 475, 555 and 635 nm solid state LED lights (Lumencor Aura III light engine) and bandpass filters to excite DAPI, Cellmask Green, Alexa568 and Cellmask Red, respectively. Images had pixel sizes of 0.68 x 0.68 microns. Three images per field were acquired at a z-interval of 4 microns. Then, best focus intensity projections were generated from the z-stack. Segmentation was carried out separately on maximum intensity projections of

Cellmask channels using CellPose 1.0, and masks were used to generate outlines and for intensity quantification. Figures were assembled with Adobe Illustrator.

## References


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