Antibody Characterization Report for Slo3

YCharOS Antibody Characterization Report

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Target:

Short protein name used in this report: Slo3

Recommended protein name: Potassium channel subfamily U member 1

Alternative protein names: Calcium-activated potassium channel subunit alpha-3, Calcium-activated potassium channel, subfamily M subunit alpha-3, KCa5, Slowpoke homolog 3

Gene name: *KCNU1*

UniProt ID: human: A8MYU2, mouse: O54982

We are a third-party organization with the mission to characterize commercial antibodies for all human protein through open science[1]. In this study, we characterized five Slo3 commercial antibodies for western blot using a standardized experimental protocol[2,3] based on comparing read-outs in knockout (KO) and isogenic parental controls (wild-type; WT). We identified well-performing antibodies and encourage readers to use this report as a guide to select the most appropriate antibody for their specific needs. Evidence of appropriate *KCNU1* expression in testis is shown elsewhere[4]. WT and *KCNU1* KO testis/epididiymis tissues from mouse were kindly donated by Dr. Celia Santi at Washington University School of Medicine, USA. Western blots (Figure 1) show that the KO used did not lead to a complete loss of the Slo3 protein. Additionally, lysates from a HEK 293 *KCNU1* overexpression cell line was obtained from an industrial collaborator to test the antibodies' ability to recognize human Slo3 (Figure 2).

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 Slo3 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications	Immunogen sequence
Abcam	ab94586*	GR50096-16	AB_2249598	monoclonal	N2/16	mouse	1.00	Wb	mouse Slo3 aa 1050 - Ct
ABclonal	A14967	0110620101	AB_2761850	polyclonal	-	rabbit	2.01	Wb	human Slo3 aa 920-1149
GeneTex	GTX42005*	822105914	AB_10733191	monoclonal	S2-16	mouse	1.00	Wb, IF	mouse Slo3 aa 1052-1121
Novus Biologicals	NBP3-05611	0110620101	AB_3094815	polyclonal	-	rabbit	1.178	Wb	human Slo3 aa 920-1149
Thermo Fisher Scientific	MA5-27598*	XB3464751	AB_2735365	monoclonal	S2-16	mouse	1.00	Wb, IF	mouse Slo3 aa 1052-1121

Wb=western blot, IHC= immunohistochemistry, IF=immunofluorescence, *=monoclonal antibody, aa=amino acid, Ct=C-terminus

Note 1: ab94586, GTX42005* and MA5-27598* appear to be the same monoclonal antibody. A14967 and NBP3-05611 appear to be the same polyclonal antibody.*

Note 2: Mammalian expression plasmid of anti-KCNU1/Slo3 (mouse) derived from hybridoma N2/16 is available at Addgene: (plasmid #114541; n2t.net/addgene:114541).

Materials and methods

Antibodies

All the Slo3 antibodies tested are listed in Table 1. Peroxidase-conjugated goat anti-rabbit and anti-mouse are from Thermo Fisher Scientific (cat. number 65-6120 and 62-6520).

Antibody screening by western blot

Western blots were performed as described in our standard operating procedure[3]. Mouse testis/epididymis from WT and *KCNU1* KO were lysed with 10 strokes of a homogenizer in HEPES buffer (10mM HEPES, pH 7.4) supplemented with 1x protease inhibitor cocktail mix (MilliporeSigma, cat. number P8340). Lysates were centrifuged at 2500x *g* for 5 min at 4°C and post-nuclear supernatants were collected. Supernatants were incubated for 30 min at 4°C with Triton X-100 added at 1% final concentration. Supernatants were spun at ~110,000x *g* for 30 min at 4°C. Equal protein aliquots of the freshly prepared supernatants from WT and KO were analyzed by SDS-PAGE and western blot. BLUelf prestained protein ladder from GeneDireX (cat. number PM008-0500) was used.

Western blots were performed with precast midi 4-20% Tris-Glycine polyacrylamide gels from Thermo Fisher Scientific (cat. number WXP42012BOX) ran with Tris/Glycine/SDS buffer from Bio-Rad (cat. number 1610772), loaded in Laemmli loading sample buffer from Thermo Fisher Scientific (cat. number AAJ61337AD) and transferred on nitrocellulose membranes. Proteins on the blots were visualized with Ponceau S staining (Thermo Fisher Scientific, cat. number BP103-10) which is scanned to show together with individual western blot. Blots were blocked with 5% milk for 1 hr, and antibodies were incubated O/N at 4°C with 5% milk in TBS with 0,1% Tween 20 (TBST) from Cell Signaling (cat. number 9997). 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 were incubated with Pierce ECL from Thermo Fisher Scientific (cat. number 32106) prior to detection with the iBright™ CL1500 Imaging System from Thermo Fisher Scientific (cat. number A44240).

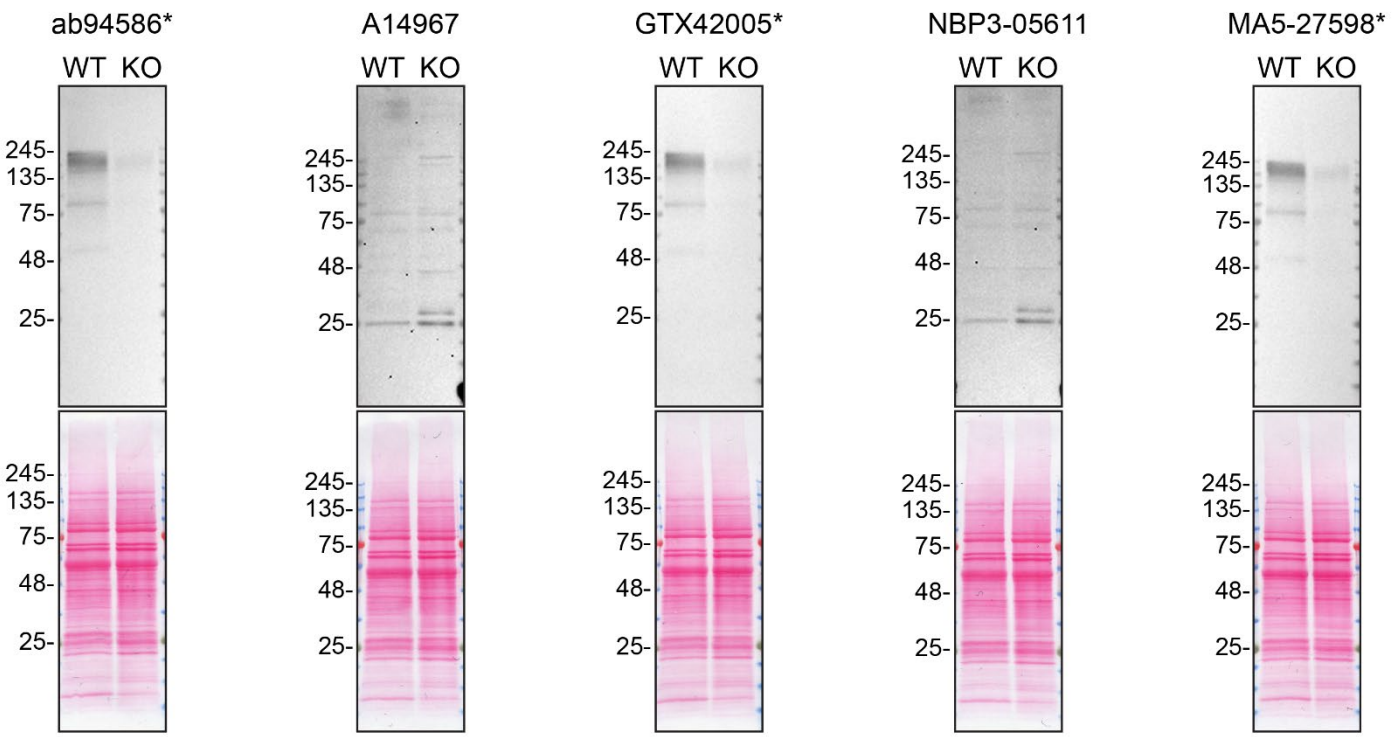

Figure 1: Slo3 antibody screening by western blot on testis/epididymis from mouse

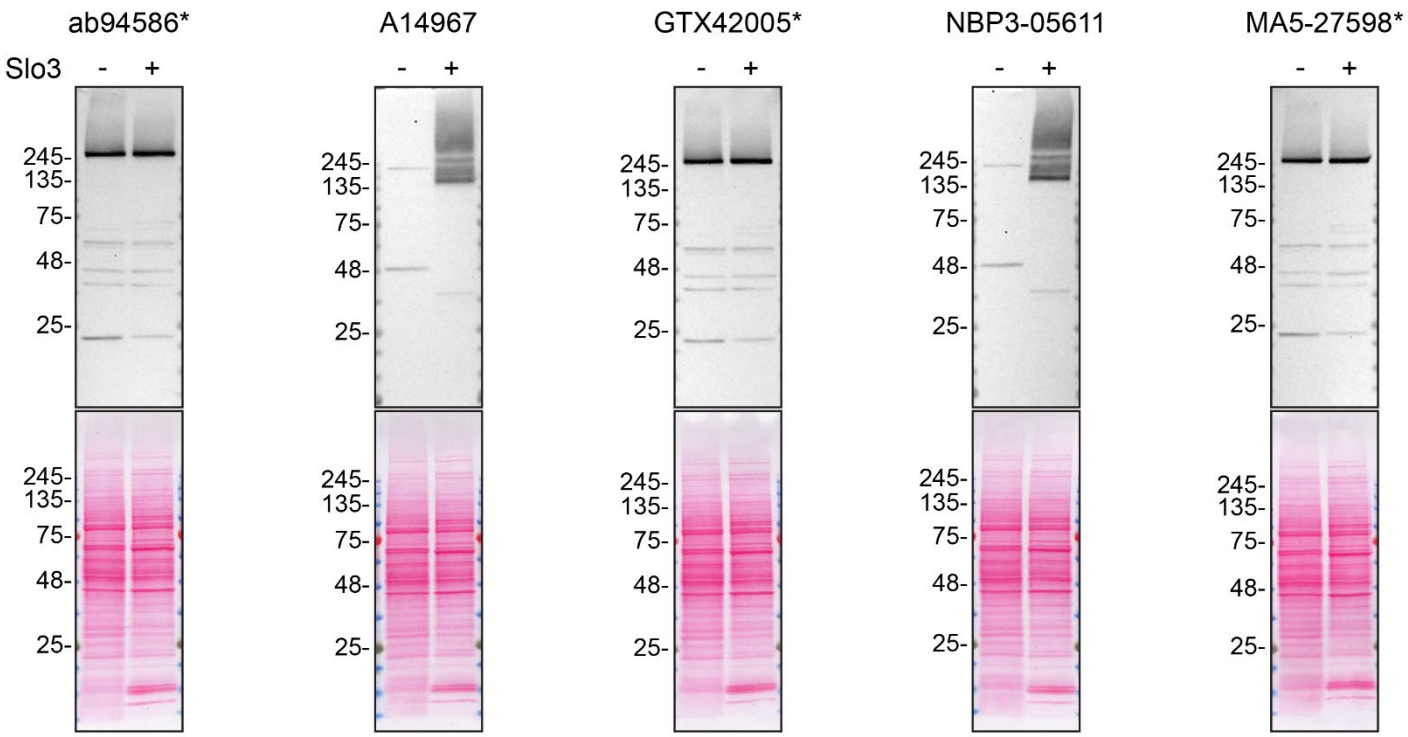

Figure 2: Slo3 antibody screening by western blot on HEK 293 *KCNU1* overexpression cell line

Figure 1: Slo3 antibody screening by western blot on testis/epididymis from mouse.

Lysates of mouse testis/epididymis from WT and *KCNU1* KO were prepared fresh, and 35 µg of protein were processed for western blot with the indicated Slo3 antibodies. The Ponceau stained transfers of each blot are shown. All antibodies were used at 1/1000. Predicted band size: 129.5 kDa. *=monoclonal antibody

Figure 2: Slo3 antibody screening by western blot on HEK 293 *KCNU1* overexpression cell line.

40 µg of RIPA lysates from HEK 293 control (-) and overexpressing *KCNU1* (+) were processed for western blot with the indicated Slo3 antibodies. The Ponceau stained transfers of each blot are shown. All antibodies were used at 1/1000. Predicted band size: 129.5 kDa. *=monoclonal antibody

References

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