Antibody Characterization Report for Secreted frizzled-related protein 1

YCharOS Antibody Characterization Report

Author(s): Riham Ayoubi[1], Peter S. McPherson[1*] and Carl Laflamme[1*]

[1] Tanenbaum Open Science Institute, Structural Genomics Consortium, Montreal Neurological Institute, McGill University, Montreal, Canada
* Corresponding authors: carl.laflamme@mcgill.ca, peter.mcpherson@mcgill.ca

Target:

Recommended protein name: Secreted frizzled-related protein 1

Alternative name: Secreted apoptosis-related protein 2

Gene name: SFRP1

Uniprot: Q8N474

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 Secreted frizzled-related protein 1. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Secreted frizzled-related protein 1 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. A549 was selected based on evidence of appropriate Secreted frizzled-related protein 1 gene expression determined using DepMap [3]. An A549 *SFRP1* KO line is available at Abcam and was used in this study. It is important to note that our data (Figure 1) indicates that the 52 bp deletion in exon 2 from the A549 *SFRP1* KO leads to a truncated Secreted frizzled-related protein 1 rather than a complete loss of the protein. The truncated Secreted frizzled-related protein 1 is not secreted out of the cell (Figure 2), in contrast with the WT form of the protein.

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 Secreted frizzled-related protein 1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Thermo	MA5-32675**	WJ3417745	AB_2809952	recombinant-mono	JA11-68	rabbit	1.00	Wb
Thermo	MA5-38193**	WJ3417799B	AB_2898110	recombinant-mono	ARC1683	rabbit	0.88	Wb
GeneTex	GTX24193	822102161	AB_370619	polyclonal	-	rabbit	1.00	Wb, IF, IHC
GeneTex	GTX102371	39911	AB_1951886	polyclonal	-	rabbit	0.33	Wb
ABclonal	A9656**	4000001683	AB_2863750	recombinant-mono	ARC1683	rabbit	0.88	Wb
ABclonal	A2911	31570101	AB_2764730	polyclonal	-	rabbit	2.68	Wb, IHC
Bio-Techne	AF1384	IRQ1020021	AB_2285831	polyclonal	-	goat	0.20	Wb, IF, IHC
Abcam	ab4193	GR3345186-4	AB_304357	polyclonal	-	rabbit	1.00	Wb, ICC/IF
Abcam	ab126613**	GR3350102-3	AB_11128257	recombinant-mono	EPR7003	rabbit	0.41	Wb, IHC-P
Abcam	ab267466**	GR3321068-3	AB_2904616	recombinant-mono	EPR23092-253	rabbit	0.46	Wb,IP, Flow Cyt
Abcam	ab84003	GR42188-1	AB_10670402	polyclonal	-	rabbit	1.00	Wb

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

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
Abcam	ab275463	CVCL_0023	A549	WT
Abcam	ab277906	CVCL_B2Q1	A549	*SFRP1* KO

Figure 1: Secreted frizzled-related protein 1 antibody screening by immunoblot.

A549 WT and *SFRP1* KO cells were treated with Brefeldin A at 3.0 µg/ml for 18 hrs. 50 µg of total lysates from treated and non-treated cells were processed for immunoblot with the indicated Secreted frizzled-related protein 1 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: MA5-32675 at 1/500; MA5-38193 at 1/500; GTX24193 at 1/1000; GTX102371 at 1/1000; A9656 at 1/1000; A2911 at 1/1000; AF1384 at 1/500; ab4193 at 1/500; ab126613 at 1/1000; ab267466 at 1/1000; ab84003 at 1/1000. Secreted frizzled-related protein 1 predicted band size: 35 kDa. **=recombinant antibody

Figure 2: Secreted frizzled-related protein 1 antibody screening by immunoblot on culture media.

100 µg of protein from concentrated culture media were processed for immunoblot with the indicated Secreted frizzled-related protein 1 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: MA5-32675 at 1/500; MA5-38193 at 1/500; GTX24193 at 1/1000; GTX102371 at 1/1000; A9656 at 1/1000; A2911 at 1/1000; AF1384 at 1/200; ab4193 at 1/500; ab126613 at 1/1000; ab267466 at 1/1000; ab84003 at 1/1000. Secreted frizzled-related protein 1 predicted band size: 35 kDa. **=recombinant antibody

Figure 3: Secreted frizzled-related protein 1 antibody screening by immunoprecipitation on culture media.

Immunoprecipitation was performed on concentrated culture media using 1.0 µg of the indicated Secreted frizzled-related protein 1 antibodies pre-coupled to either protein G or protein A magnetic beads. Samples were washed and processed for immunoblot with the indicated Secreted frizzled-related protein 1 antibodies. For immunoblot, MA5-38193 was used at 1/500, A9656 at 1/1000 and ab126613 at 1/1000. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate. LC= light chain **=recombinant antibody.

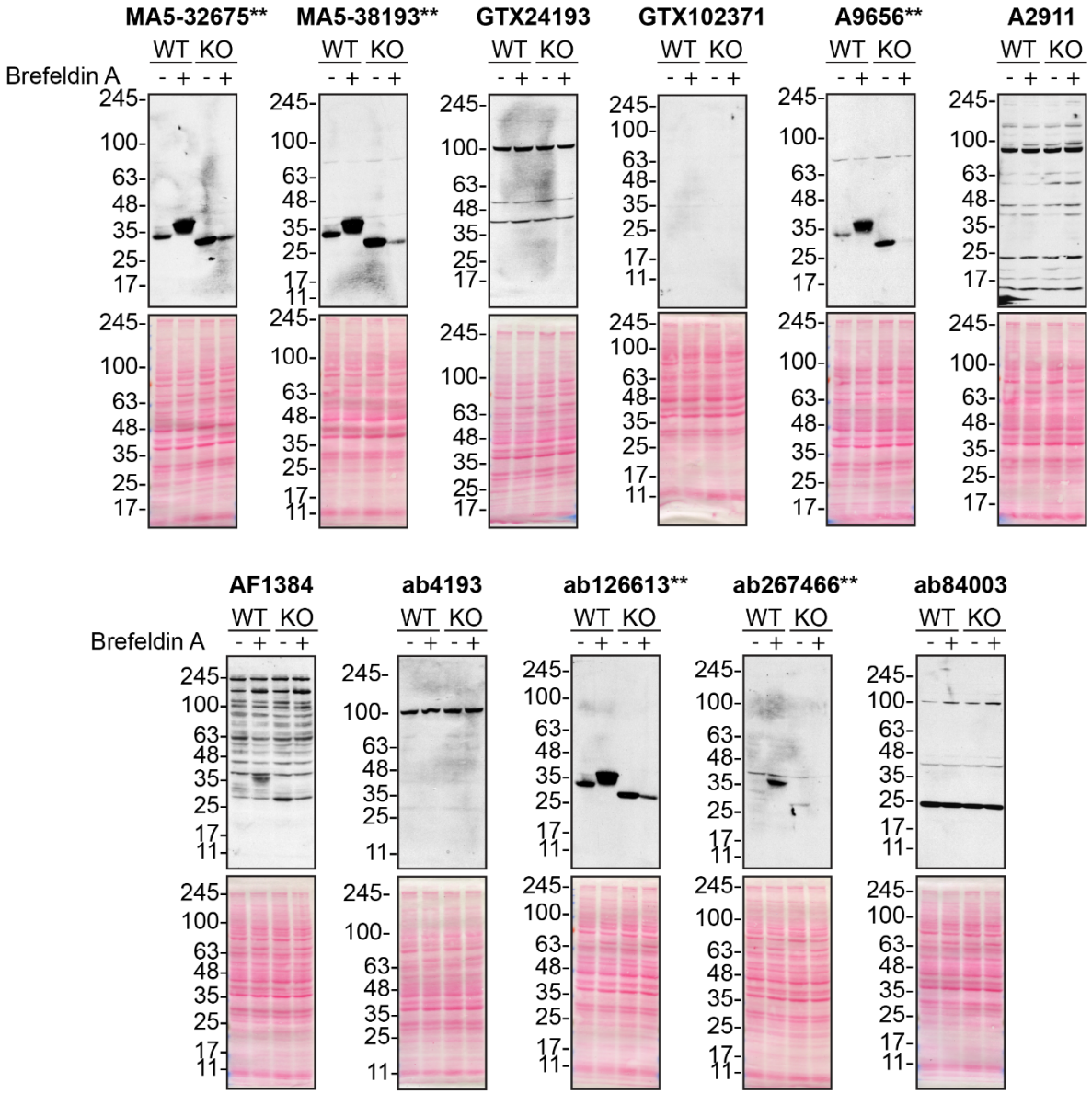

Figure 1: Inhibition of Secreted frizzled-related protein 1 secretion by Brefeldin A

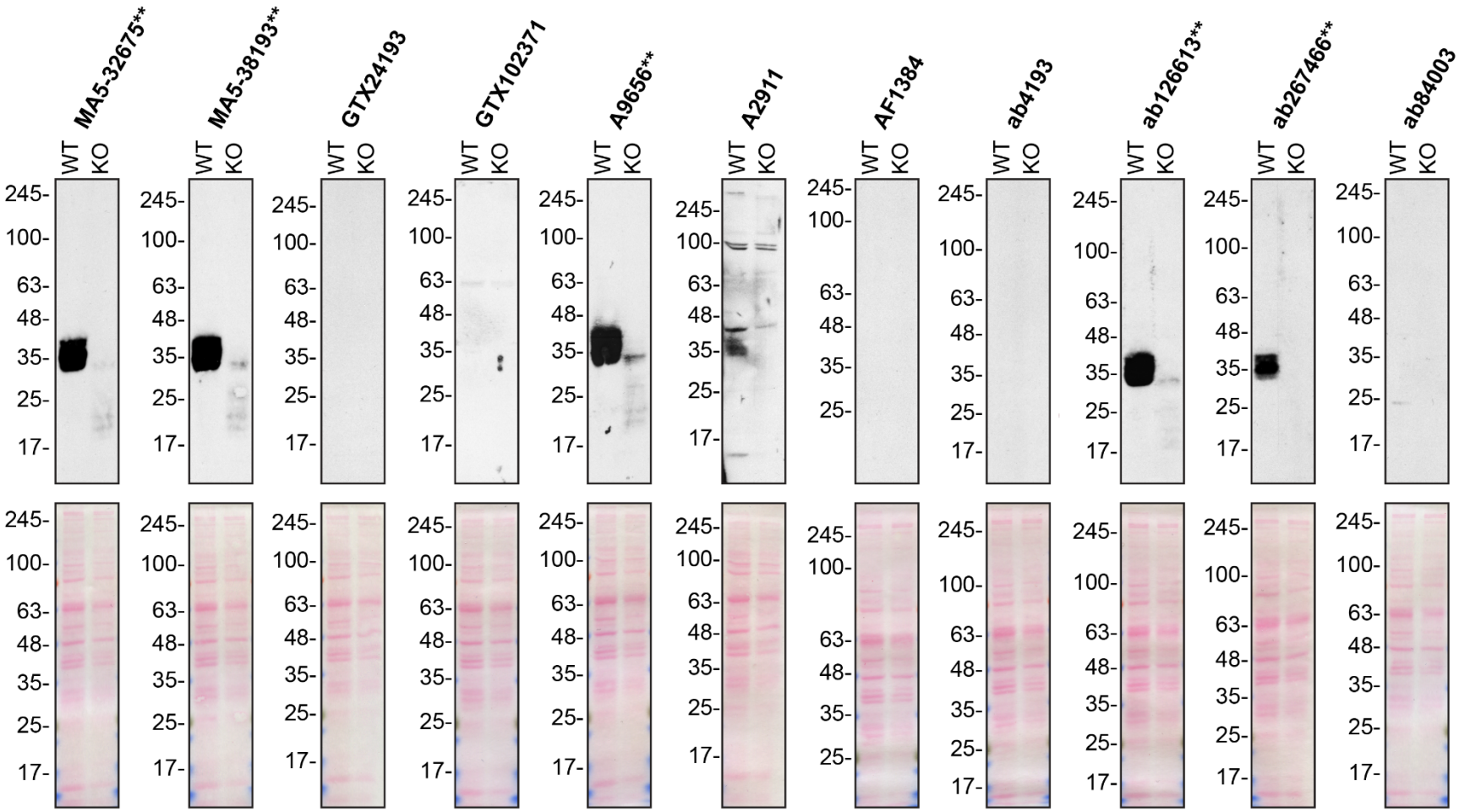

Figure 2: Secreted frizzled-related protein 1 antibody screening by immunoblot on culture media

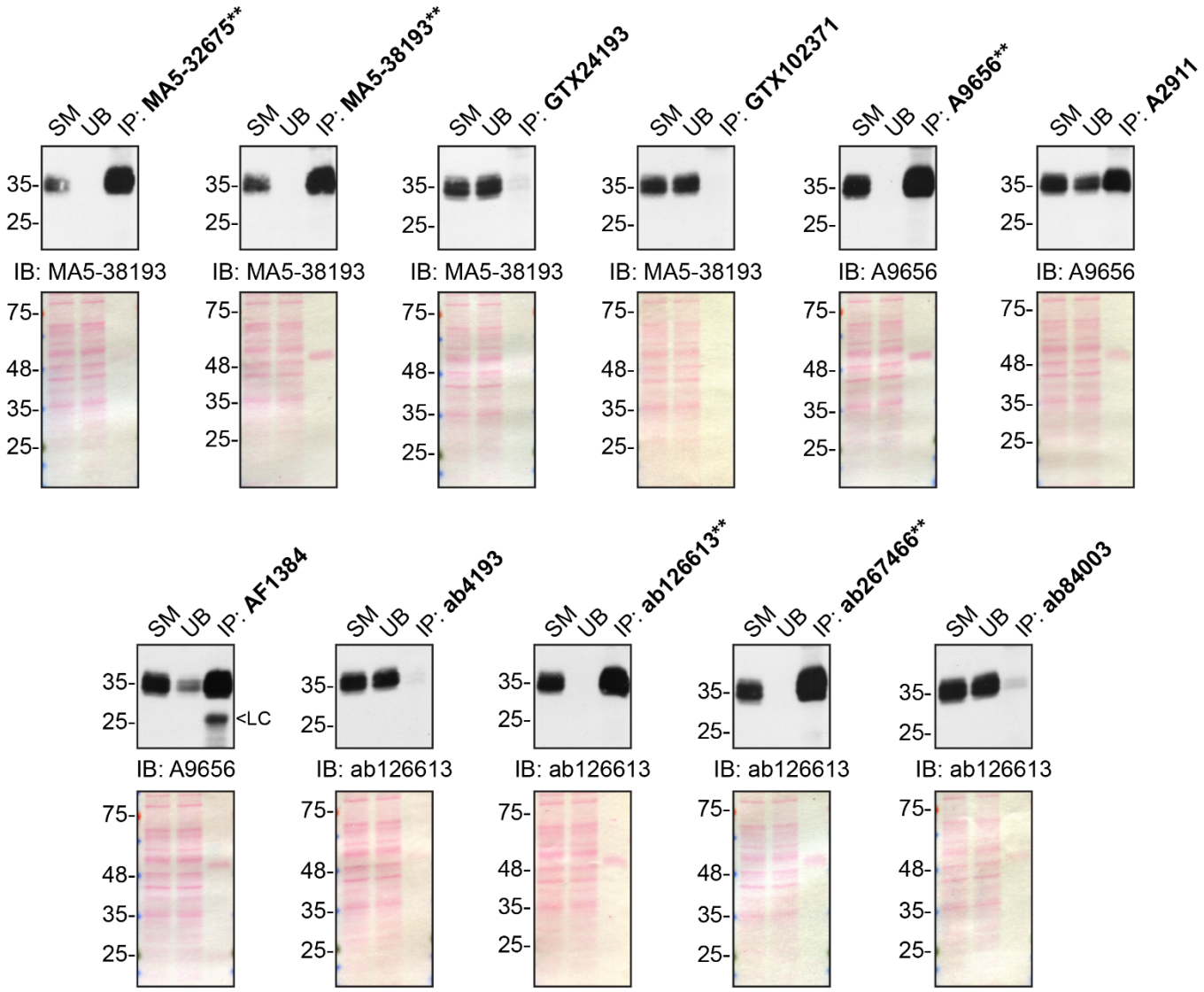

Figure 3: Secreted frizzled-related protein 1 antibody screening by immunoprecipitation on culture media

Materials and methods

Antibodies

All Secreted frizzled-related protein 1 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).

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). Cells were starved in DMEM high glucose containing L-glutamate and penicillin/ streptomycin.

Lysates from Brefeldin A-treated cells

A549 cells were treated with 0.3 µg/ml of Brefeldin A from Thermo Fisher Scientific (cat. number 00- 4506-51). A549 cells (WT and *SFRP1* KO) treated and non-treated 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.

Collection of culture media

A549 WT and *SFRP1* KO cells were washed 3x with PBS and starved for ~18 hrs. Culture media were collected and centrifuged for 10 min at 500 x g to eliminate cells and larger contaminants, then for 10 min at 4500 x g to eliminate smaller contaminants. Culture media were concentrated by centrifuging at 4000 x g for 10min using Amicon Ultra-15 Centrifugal Filter Units with a membrane NMWL of 10kDa (MilliporeSigma cat. number UFC901024). Immunoblots were performed as described above.

Antibody screening by immunoblot

Immunoblots were performed as described in our standard operating procedure [4]. Large 8-16% gradient polyacrylamide gels were used 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 on culture media

Immunoprecipitation was performed as described in our SOP for immunoprecipitation [5]. Antibody-bead conjugates were prepared by adding 1 µg of antibody to 500 ul of Pierce IP Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together with 30µl of Dynabeads protein A- (for rabbit antibodies) or protein G- (for goat antibodies) from Thermo Fisher Scientific (cat. number 10002D and 10004D, respectively). Pierce IP Lysis Buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol) 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.

Starved A549 WT culture media were concentrated as described above. 1ml aliquots at 0.5 mg/ml of protein 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 IP Lysis Buffer and processed for SDS-PAGE and immunoblot on 8-16% polyacrylamide gels. Prot-A:HRP (MilliporeSigma, cat. number P8651) was used as a secondary detection system at a dilution of 0.4 µg/ml.

References

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2. Laflamme, C., et al., *Implementation of an antibody characterization procedure and application to the major ALS/FTD disease gene C9ORF72.* Elife, 2019. **8** DOI: 10.7554/eLife.48363.
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4. Ayoubi, R., P.S. McPherson, and C. Laflamme, *Antibody Screening by Immunoblot.* 2021 DOI: https://doi.org/10.5281/zenodo.5717510.
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