Antibody Characterization Report for Macrophage colony-stimulating factor 1 (CSF-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
[2] Early Drug Discovery Unit (EDDU), Montreal Neurological Institute, McGill
* Corresponding authors: carl.laflamme@mcgill.ca, peter.mcpherson@mcgill.ca

Target:

Recommended protein name: Macrophage colony-stimulating factor 1

Short recommended protein name: CSF-1

Alternative protein names: M-CSF, MCSF

Gene name: CSF1

Uniprot: P09603

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 CSF-1. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for CSF-1 by immunoblot (Western blot) and immunoprecipitation. U87-MG was selected based on evidence of appropriate CSF-1 gene expression determined using DepMap [3]. U87-MG was modified with CRISPR/Cas9 to knockout the corresponding *CSF1* gene and the KO cell line is available at Abcam.

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 CSF-1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Abcam	ab52864**	GR3302408-1	AB_880690	recombinant-mono	EP1179Y	rabbit	0.73	Wb,IP,IF
Abcam	ab99178	GR3345362-2	AB_11143555	polyclonal	-	rabbit	0.30	Wb
Abcam	ab233387**	GR3247123-3	AB_2892678	recombinant-mono	EPR20948	rabbit	0.45	Wb,IF
Bio-Techne	NBP2-67490**	HN1102	AB_2920822	recombinant-mono	SU0413	rabbit	1.00	Wb,IP,IF
GeneTex	GTX81735	822104516	AB_11173351	polyclonal	-	rabbit	2.00	Wb,IF
Thermo Fisher Scientific	MA5-15599*	WJ3417324	AB_10986835	monoclonal	2D10	mouse	not provided	other
Thermo Fisher Scientific	MA5-32139**	WA3171002	AB_2809429	recombinant-mono	SU0413	rabbit	1.00	Wb,IF
Bio-Techne	MAB616*	BLF0920051	AB_2084934	monoclonal	21113	mouse	1.00	Wb
Bio-Techne	MAB216*	JG0321071	AB_2085064	monoclonal	26730	mouse	1.00	Wb
ABclonal	A1627	209540301	AB_2763687	polyclonal	-	rabbit	1.86	Wb
Aviva Systems Biology	ARP44329	QC25309-090430	AB_938216	polyclonal	-	rabbit	0.50	Wb
Aviva Systems Biology	ARP44330	QC74491-200210	AB_10640832	polyclonal	-	rabbit	0.50	Wb
Aviva Systems Biology	ARP44331	QC61453-43546	AB_10643950	polyclonal	-	rabbit	0.50	Wb,IF
Developmental Studies Hybridoma Bank	CPTC-CSF1-1*	2/23/22	AB_2920823	monoclonal	-	rabbit	0.02	other

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
ATCC	HTB-14	CVCL_0022	U87-MG	WT
Abcam	-	-	U87-MG	*CSF1* KO

Figure 1: CSF-1 antibody screening by immunoblot on culture media.

Cell lines (WT and *CSF1 KO*) were cultured in serum free media. Media was collected and concentrated. Then, 25 µg of protein from concentrated culture media were processed for immunoblot with the indicated CSF-1 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: ab52864** at 1/2000, ab99178 at 1/300, ab233387** at 1/2000, NBP2-67490** at 1/500, GTX81735 at 1/1000, MA5-15599* at 1/1000, MA5-32139** at 1/1000, MAB616* at 1/1000, MAB216* at 1/1000, A1627 at 1/3000, ARP44329 at 1/500, ARP44330 at 1/500, ARP44331 at 1/500, CPTC-CSF1-1* at 1/19. CSF-1 predicted band size: 60 kDa. *=monoclonal antibody; **=recombinant antibody.

Figure 2: CSF-1 antibody screening by immunoprecipitation on culture media.

Immunoprecipitation was performed on 400 µg of concentrated culture media using 2.0 µg of the indicated CSF-1 antibodies pre-coupled to either protein G or protein A magnetic beads. Samples were washed and processed for immunoblot with the indicated CSF-1 antibodies. For immunoblot, ARP44329 was used at 1/500. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitated; *=monoclonal antibody; **=recombinant antibody.

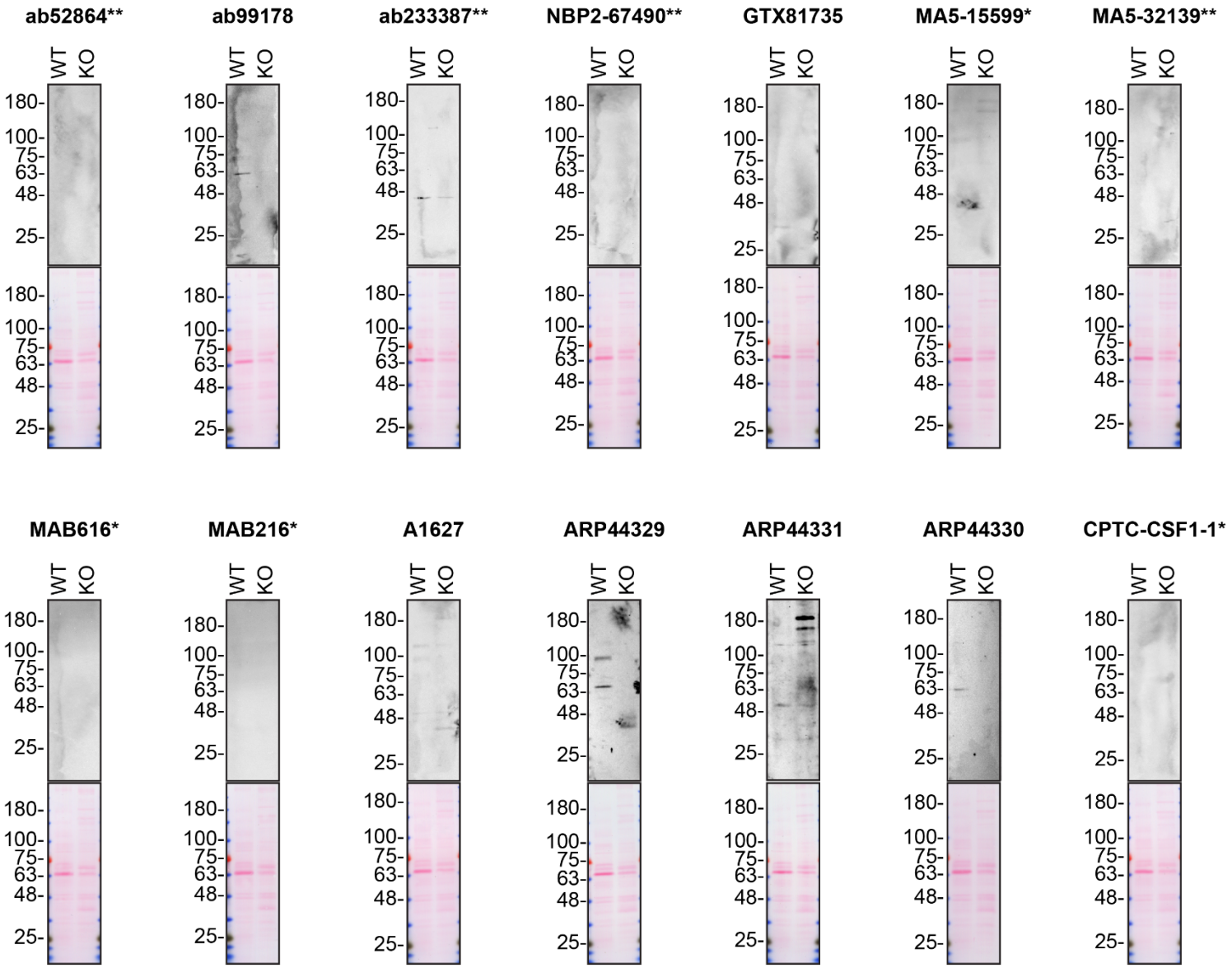

Figure 1: CSF-1 antibody screening by immunoblot on culture media

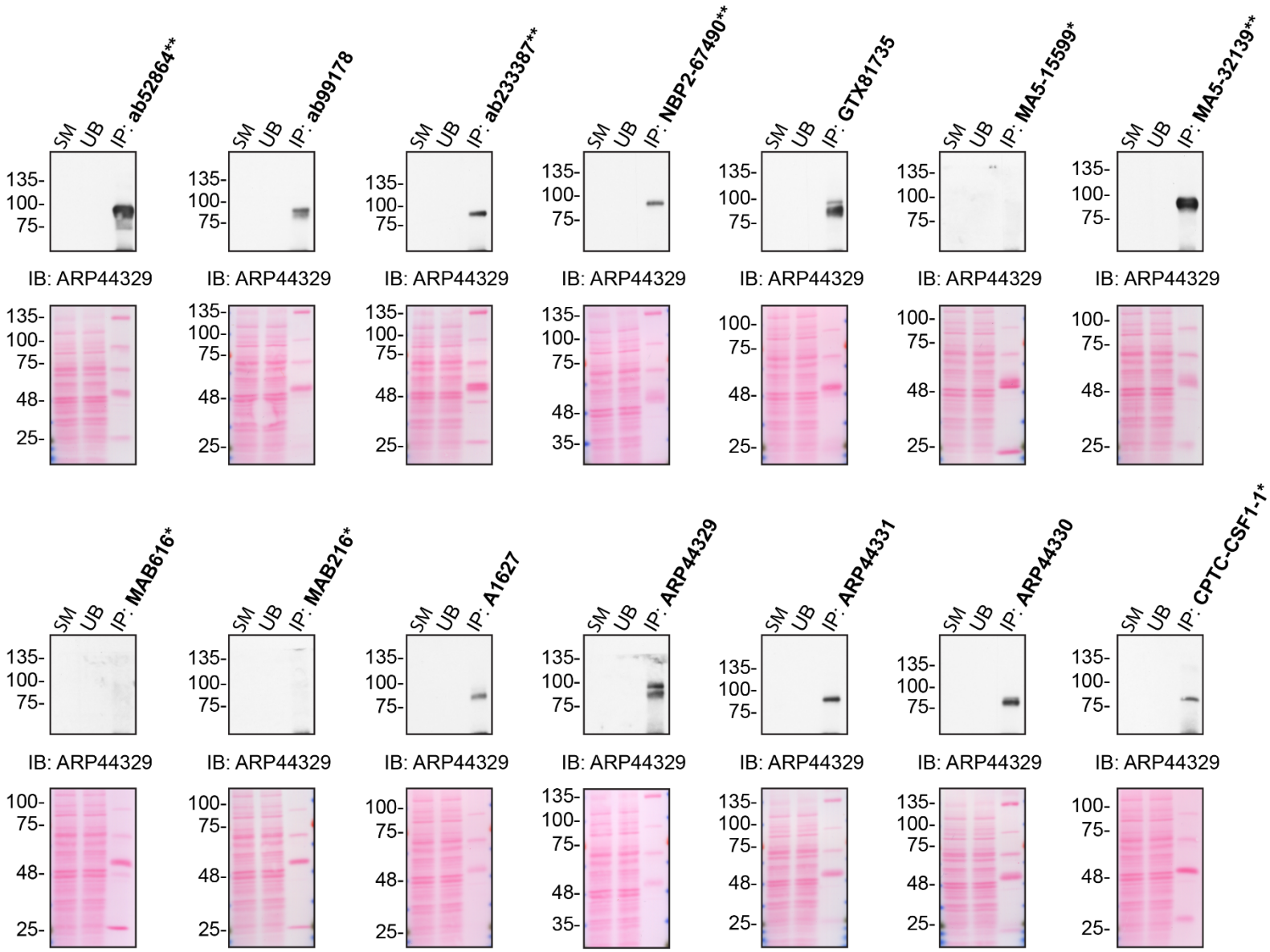

Figure 2: CSF-1 antibody screening by immunoprecipitation on culture media

Materials and methods

Antibodies

All CSF-1 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).

CRISPR/Cas9 genome editing

Cell lines used are listed in Table 2. U87-MG *CSF1* KO clone was generated with low passage cells. Guide RNA sequences used to KO *CSF1* are TCCAGTGTGCTACCTTAAGA and GCACAATGGCGATGGCATTG.

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.

Collection of culture media

U87-MG WT and *CSF1* 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 30min using Amicon Ultra-15 Centrifugal Filter Units with a membrane NMWL of 10kDa (MilliporeSigma cat. number UFC901024).

Antibody screening by immunoblot using culture media

Immunoblots were performed as described in our standard operating procedure [5]. Large 4-15% 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 using culture media

Immunoprecipitation was performed as described in our SOP for immunoprecipitation [6]. Antibody-bead conjugates were prepared by adding 2 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 Dynabeads protein G- (for mouse 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 U87-MG WT culture media were concentrated as described above. 1ml aliquots at 0.4 mg/ml of protein 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 IP Lysis Buffer and processed for SDS-PAGE and immunoblot on 4-15% polyacrylamide gels.

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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6. Ayoubi, R., et al., *Antibody screening by Immunoprecitation.* 2021 DOI: https://doi.org/10.5281/zenodo.5717516.