antibodies.com

Datasheet for ABIN7300570 anti-CSF2RA antibody (Center)

1 Validation

1

Image



Overview

Quantity:	100 μL
Target:	CSF2RA
Binding Specificity:	Center
Reactivity:	Human, Mouse, Rat
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This CSF2RA antibody is un-conjugated
Application:	Western Blotting (WB)

Product Details

Immunogen:	KLH-conjugated synthetic peptide encompassing a sequence within the center region of human CD116.
Specificity:	Recognizes endogenous levels of CD116 protein.
Characteristics:	Rabbit polyclonal antibody to CD116
Purification:	The antibody was purified by immunogen affinity chromatography.

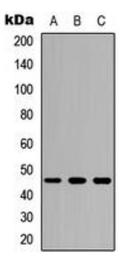
Target Details

Target:	CSF2RA
Alternative Name:	CD116 (CSF2RA Products)
Background:	CSF2R, CSF2RY, Granulocyte-macrophage colony-stimulating factor receptor subunit alpha,

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Target Details		
	GM-CSF-R-alpha, GMCSFR-alpha, GMR-alpha, CDw116, CD116	
Gene ID:	1438, 12982	
UniProt:	P15509, Q00941	
Pathways:	JAK-STAT Signaling	
Application Details		
Application Notes:	WB (1:500 - 1:1000)	
Restrictions:	For Research Use only	
Handling		
Format:	Liquid	
Buffer:	Liquid in 0.42 % Potassium phosphate, 0.87 % Sodium chloride, pH 7.3, 30 % glycerol, and 0.01 % sodium azide.	
Preservative:	Sodium azide	
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.	
Storage:	-20 °C	
Storage Comment:	Shipped at 4°C. Upon delivery aliquot and store at -20°C for one year. Avoid freeze/thaw cycles.	
Expiry Date:	12 months	

Images



Western Blotting

Image 1. Western blot analysis of CD116 expression in HEK293T (A), Raw264.7 (B), H9C2 (C) whole cell lysates.

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Successfully validated (Western Blotting (WB))

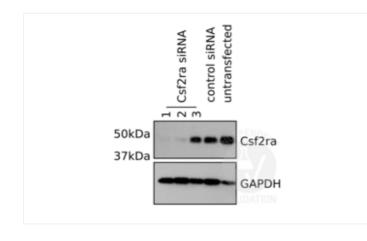
by Instituto de Parasitología y Biomedicina López-Neyra Report Number: 100104 Date: Dec 23 2016

Target:	CSF2RA
Method validated:	Western Blotting (WB)
Positive Control:	primary mouse microglia
Negative Control:	siRNA knock-down of CSF2RA in primary mouse microglia
Notes:	Passed. ABIN2705734 specifically recognizes Csf2ra in primary mouse microglia lysates.
Primary Antibody:	ABIN2705734
Secondary Antibody:	anti-rabbit HRP-conjugated antibodies (DakoCytomation, P0448, lot 00055814)
Protocol:	 Primary mouse microglia are grown in DMEM (Invitrogen), supplemented with 10% foetal bovine serum (Gibco), 10% horse serum and penicillin and streptomycin (Gibco), at 37°C and 5% CO2 in a 12-well dish. Transfect cells with 0.05nmol mouse CSF2RA Silencer Select siRNA (ThermoFisher Scientific, ASO1094M, ASO10991 and ASO109DF, lot AMO04BXD) using Lipofectamine 300 (Invitrogen) following the manufacturer's instructions. Lyse cells in 25µl per well cold lysis buffer (10mM Tris-HCI pH 8.0, 150mM NaCl, 1% Nonide P40, 1mM EDTA, 10mM NaF, 1mM Na₃VO₄, protease inhibitors (Sigma). Determine total protein content of the lysates using Bradford assay (Bio-Rad, 500-0006, lot 111832). Denature 5µg total protein for 5min at 95°C in 5µl Laemmli SDS sample buffer and subsequently separate them on a denaturing, freshly cast 10% SDS-PAGE for 1h at 140V. Transfer proteins onto PVDF membrane (Pall Life Sciences, 75696G, lot T03225) with a semi-dry Western blotting system for 50min at 60mA. Block the membrane with TBST (TBS, 0.1% Tween) containing 5% milk ON at 4°C. Incubation with primary rabbit anti-CSF2RA antibody (antibodies-online, ABIN2705734, lot CP1D10A) diluted 1:500 in TBST for 1h at RT. Wash membrane 5x 5min with TBST. Incubation with anti-rabbit horseradish peroxidase-conjugated secondary antibodies (DakoCytomation, P0448, lot 00055814) diluted 1:2000 in TBST containing 5% milk for 1h at RT. Wash membrane 5x 5min with TBST and 3x 5min with TBS. Reveal protein bands using ECL Prime Western Blotting Detection Reagent (GE Healthcare,

lot 79040074) on an AGFA Health Care Curix60-developer.

	 Strip membranes using RestoreTM Western Blot Stripping Buffer (Thermo Scientific, 21059, lot NA 164269) and subsequently incubate with a GAPDH loading control antibody (Sigma, G9545, lot 049K4787) diluted 1:10000 and with anti-rabbit horseradish peroxidase-conjugated secondary antibodies (DakoCytomation, P0448, lot 00055814) diluted 1:2000. Wash and reveal the protein bands using self-made ECL Western Blotting Detection Reagent with Curix RP2 Plus medical X-ray films (AGFA Health Care, ENKMV, lot 79040074) on an AGFA Health Care Curix60-developer.
Experimental Notes:	The RND3 antibody ABIN2705734 reveals a protein of the expected molecular weight of mouse Csf2ra in lysates of primary mouse microglia. The protein band's intensity decreases upon knockdown with two of the three utilized siRNAs.

Image for Validation report #100104



Validation image no. 1 for anti-Colony Stimulating Factor 2 Receptor, Alpha, Low-Affinity (Granulocyte-Macrophage) (CSF2RA) (Center) antibody (ABIN2705734)

Primary mouse microglia were transfected with Csf2ra siRNA and Csf2ra was revealed using ABIN2705734 as described in the protocol section (lanes 1, 2, and 3). Lysates from cells transfected with scrambled siRNA (lane 4) and untransfected cells (lane 5) served as negative controls.

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