antibodies - online.com







anti-TIAM1 antibody (AA 1-378)



Validation



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UniProt:

Quantity:	50 μg
Target:	TIAM1
Binding Specificity:	AA 1-378
Reactivity:	Mouse
Host:	Sheep
Clonality:	Polyclonal
Conjugate:	This TIAM1 antibody is un-conjugated
Application:	Western Blotting (WB)
Product Details	
Product Details Immunogen:	AA 1-378
	AA 1-378 IgG
Immunogen:	
Immunogen: Isotype:	IgG
Immunogen: Isotype: Characteristics:	IgG

Q13009

Application Details

Application Notes:	Optimal dilutions should be determined by each laboratory for each application.	
Restrictions:	For Research Use only	
Handling		
Format:	Lyophilized	
Reconstitution:	100 μL of PBS, pH 7.4	
Concentration:	500 μg/mL	
Buffer:	Lyophilized from a solution containing PBS, pH 7.4	
Storage:	-20 °C	





Successfully validated (Western Blotting (WB))

by Instituto de Parasitología y Biomedicina López-Neyra

Report Number: 100102

Date: Dec 23 2016

RT.

Target:	TIAM1
Lot Number:	2016090702
Method validated:	Western Blotting (WB)
Positive Control:	primary mouse microglia, MCF-7
Notes:	Passed. ABIN1982879 specifically recognizes TIAM1 in lysates from human MCF-7 cells.
Primary Antibody:	ABIN1982879
Secondary Antibody:	anti-goat/sheep HRP-conjugated antibodies (DakoCytomation, P0449, lot 00062104)
Protocol:	 Primary mouse microglia and human MCF-7 cells 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 plate or in RPMI 1640 (Invitrogen), supplemented with 6% inactivated foetal bovine serum (Gibco), GlutaMax (Gibco), penicillin and streptomycin (Gibco), at 37°C and 5% CO2 in a 10cm dish, respectively. Lyse microglia in 25µl per well and MCF-7 cells in 500µl per dish cold lysis buffer (10mM Tris-HCl pH 8.0, 150mM NaCl, 1% Nonidet-P40, 1mM EDTA, 10mM NaF, 1mM Na_{3vo4}, containing protease inhibitors (Sigma)). Determine total protein content of the lysates using Bradford assay (Bio-Rad, 500-0006, lot 111832). Denature 30µg total protein for 5min at 95°C in in 20µl Laemmli SDS sample buffer and subsequently separate them on a denaturing, freshly cast 7.5% SDS-PAGE for 1h at 140V. Transfer proteins onto PVDF membrane (Pall Life Sciences, 75696G, lot T03225) with a wet Western blotting system for 80min at 100V. Block the membrane with TBST (TBS, 0.1% Tween) containing 5% milk ON at 4°C. Incubation with primary sheep anti-TIAM1 antibody (antibodies-online, ABIN1982879, lot 2016090702) diluted 1:500 in TBST containing 5% milk for 1h at RT. Wash membrane 5x 5min with TBST. Incubation with anti-goat/sheep horseradish peroxidase-conjugated secondary antibodies

· Wash membrane 5x 5min with TBST and 3x 5min with TBS.

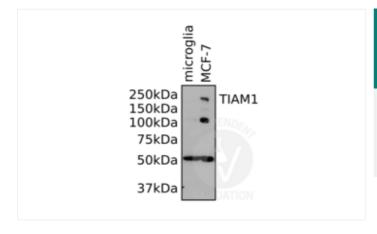
(DakoCytomation, P0449, lot 00062104) diluted 1:2000 in TBST containing 5% milk for 1h at

• Reveal protein bands using self-made ECL Western Blotting Detection Reagent with Curix RP2 Plus medical X-ray films (Agfa, ENKMV, lot 79040074) on an Agfa- Curix60-developer.



The TIAM1 antibody ABIN1982879 reveals a protein of the expected molecular weight of 177kDa in human MCF-7 lysates as verified with a second anti-TIAM1 antibody. However, note that ABIN1982879 did not recognize Tiam1 in primary mouse microglia extracts.

Image for Validation report #100102



Validation image no. 1 for anti-T-Cell Lymphoma Invasion and Metastasis 1 (TIAM1) (AA 1-378) antibody (ABIN1982879)

ABIN1982879 was used to reveal TIAM1 in lysates from human MCF-7 (lane 2) cells and mouse primary mouse microglia (lane 1) as described in the protocol.