Datasheet for ABIN1537116

anti-MR1 antibody (C-Term)

3 Validations

1 Image

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Overview

Quantity:	400 µL
Target:	MR1
Binding Specificity:	AA 312-341, C-Term
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This MR1 antibody is un-conjugated
Application:	Western Blotting (WB)

Product Details

Immunogen:	This MR1 antibody is generated from rabbits immunized with a KLH conjugated synthetic peptide between 312-341 amino acids from the C-terminal region of human MR1.
Clone:	RB37199
Isotype:	IgG
Purification:	This antibody is purified through a protein A column, followed by peptide affinity purification.

Target Details

Target:	MR1
Alternative Name:	MR1 (MR1 Products)
Background:	MR1 has antigen presentation function. Involved in the development and expansion of a small

Target Details

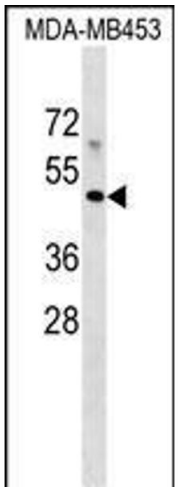
	population of T cells expressing an invariant T cell receptor alpha chain called mucosal-associated invariant T cells (MAIT). MAIT cells are preferentially located in the gut lamina propria and therfore may be involed in monitoring commensal flora or serve as a distress signal. Expression and MAIT cell recognition seem to be ligand-dependent.
Molecular Weight:	39366
Gene ID:	3140
NCBI Accession:	NP_001181928, NP_001181929, NP_001181964, NP_001522
UniProt:	Q95460
Pathways:	Regulation of Leukocyte Mediated Immunity, Positive Regulation of Immune Effector Process, Production of Molecular Mediator of Immune Response, Cancer Immune Checkpoints

Application Details

Application Notes:	WB: 1:1000
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	Purified polyclonal antibody supplied in PBS with 0.09 % (W/V) 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:	4 °C,-20 °C
Storage Comment:	MR1 Antibody (C-term) can be refrigerated at 2-8 °C for up to 6 months. For long term storage, keep at -20 °C.
Expiry Date:	6 months



Western Blotting

Image 1. MR1 Antibody (C-term) (ABIN1537116 and ABIN2848960) western blot analysis in MDA-M cell line lysates (35 µg/lane).This demonstrates the MR1 antibody detected the MR1 protein (arrow).

Successfully validated (Immunocytochemistry (ICC))

by Dr. Randy Brutkiewicz Laboratory, Department of Microbiology and Immunology, Indiana University School of Medicine

Report Number: 101753

Date: Feb 20 2018

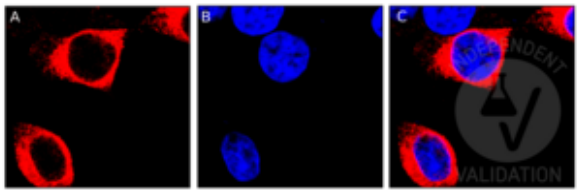
Target:	MR1
Lot Number:	SA111213CH
Method validated:	Immunocytochemistry (ICC)
Positive Control:	HEK293 cells transfected with human MR1 cDNA
Negative Control:	HEK293 cells transfected with plasmid vector only
Notes:	Passed. The MR1 antibody ABIN1537116 specifically labels the targeted antigen in HEK293 ectopically expressing human MR1 in ICC.
Primary Antibody:	ABIN1537116
Secondary Antibody:	Texas Red-conjugated donkey anti-rabbit immunoglobulin antiserum (Jackson ImmunoResearch, 711-076-152, lot 66576)
Protocol:	• Grow HEK293 cells in DMEM medium (Lonza, 12-614F, lot 0000618582) supplemented with serum (Hyclone, SH30071.03, lot AAG205460) and antibiotics (Hyclone, SV30010, lot J150013), at 37°C and 5% CO_2 dish to 70-90% confluency. • Transfect cells with pCDNA 3.1 neo (-) (Invitrogen) containing human MR1 cDNA (Genecopoeia) using Polyethylenimine (Polysciences, 23966) following the manufacturer´s instructions. • Plate cells in sterile glass-bottom 35-mm dishes coated with collagen (MatTek, P35GCol-1.5-14-C). Let cells grow to 50-80% confluency. • Wash cells d with PBS. • Fix cells with 4% paraformaldehyde for 15min at RT. • Block cells with blocking buffer (1x PBS, 5% donkey serum, 0.3% Triton X-100) for 1h atRT. • Incubate cells with primary rabbit anti-MR1 antibody (antibodies-online, ABIN1537116, lot SA111213CH) diluted 1:50 in dilution buffer (1X PBS / 1% BSA / 0.3% Triton X-100) and incubated ON at 4°C. • Wash cells 3x with PBS. • Incubate cells with Texas Red-conjugated donkey anti-rabbit immunoglobulin antiserum (Jackson ImmunoResearch, 711-076-152, lot 66576) diluted 1:50 in dilution buffer for 1h at RT. • Wash cells 3x with PBS. • To stain the nucleus, cells were immersed in PBS-containing Hoechst diluted 1:2000 in PBS

for 5min.

- Just prior to confocal analysis, place cells in mounting medium (10mM Tris pH8.5, 2% DABCO).
- Image cells on an Olympus 2 confocal/two-photon microscope imaging system using an oil immersion lens at 60×.

Experimental Notes:	Staining with ABIN1537116 shows a perinuclear pattern, suggesting MR1 localizes in the endoplasmic reticulum. No signal was detected in sample negative control tissue and the secondary antibody only control.

Image for Validation report #101753



Validation image no. 1 for anti-Major Histocompatibility Complex, Class I-Related (MR1) (AA 312-341), (C-Term) antibody (ABIN1537116)

Human MR1-expressing HEK293 cells were stained with MR1 antibody ABIN1537116 and a Texas Red-conjugated secondary antibody (red, A). For nuclear staining, cells were stained with Hoechst (blue, B). C shows both channels merged.

Validation report #102828 for Immunoprecipitation (IP)

Successfully validated (Immunoprecipitation (IP))

by Dr. Randy Brutkiewicz Laboratory, Department of Microbiology and Immunology, Indiana University School of Medicine

Report Number: 102828

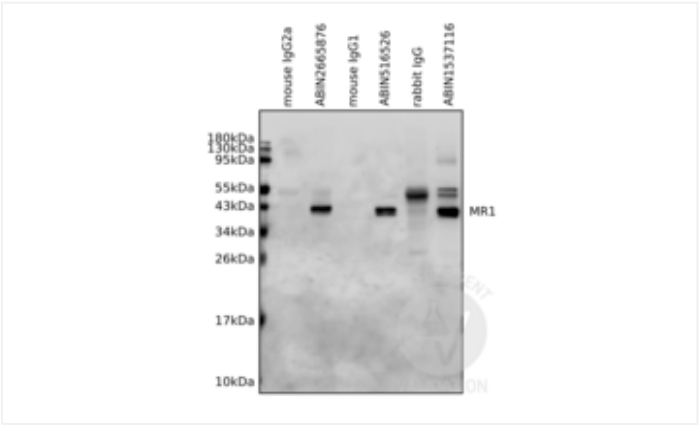
Date: Feb 20 2018

Target:	MR1
Lot Number:	SA111213CH
Method validated:	Immunoprecipitation (IP)
Positive Control:	HEK293 cells transfected with human MR1 cDNA
Negative Control:	HEK293 cells transfected with plasmid vector only
Notes:	Passed. ABIN1537116 immunoprecipitates human MR1 overexpressed by HEK293 cells.
Primary Antibody:	ABIN1537116
Secondary Antibody:	goat anti-rabbit Dye-IR800 conjugated antibody (Advansta, R-05060-250, lot 17083179)
Protocol:	• Grow HEK293 cells in DMEM medium (Lonza, 12-614F, lot 0000618582) supplemented with serum (Hyclone, SH30071.03, lot AAG205460) and antibiotics (Hyclone, SV30010, lot J150013), at 37°C and 5% CO_2 dish to 70-90% confluency. • Transfect cells with pCDNA 3.1 neo (-) (Invitrogen) containing human MR1 cDNA (Genecopoeia) using Polyethylenimine (Polysciences, 23966) following the manufacturer´s instructions. • Lyse cells in cold lysis buffer (10mM Tris pH7.4, 150mM NaCl, 0.5mM EDTA, 2% CHAPS). • Determine total protein content of the lysates using Commassie Protein Assay Reagent (Thermo Scientific, 1856209, lot NL179252). • Immobilize 100µl of protein G-conjugated Sepharose beads (Pierce, product 20399, lot RI239318) ON at 4°C with 2.5µg mouse anti-MR1 antibody (antibodies-online, ABIN2665876, lot B177559), ○ 2.5µg mouse anti-MR1 antibody (antibodies-online, ABIN516526, lot12045-5B5), ○ 2.5µg rabbit anti-MR1 antibody (antibodies-online, ABIN1537116, lot SA111213CH), ○ 2.5µg mouse IgG2a antibody (Biolegend, 400202, lot B153642), ○ 2.5µg mouse IgG1 antibody (BD, 555746, lot 3221830), or ○ 2.5µg rabbit IgG antibody (Santa Cruz Biotechnology, SC-5560, lot E0609). • Incubate 500µg of the cell lysates with 2.5µg of antibody-bead conjugate ON at 4°C. • Wash lysates 4x with PBS. • Denature beads for 5min at 95°C in 60µl Laemmli SDS sample buffer and subsequently separate them on a SDS-PAGE gel using Acrylamide/Bis Premixed (Bio-Rad, 61-0125, lot 260000477) for 2-3h at 100V.

- Transfer proteins onto PVDF membrane (Millipore, IPVH00010, lot K5AA6843U) with a Western blotting system for ON at 4°C at 150mA.
- Block the membrane with blocking buffer (2% BSA/PBS/0.05%Tween-20) for 1h at RT.
- Incubate membrane with primary rabbit anti-MR1 antibody (antibodies-online ABIN1537116, lot SA111213CH) diluted 1:1000 in blocking buffer ON at 4°C.
- Wash membrane 3x for 10min with PBS/0.05%Tween-20.
- Incubate membrane with secondary goat anti-rabbit Dye-IR800 conjugated antibody (Advansta, R-05060-250, lot 17083179) diluted 1:10000 in PBS/0.05% Tween-20 for 1h at RT.
- Wash membrane 3x for 10 min with PBS/0.05% Tween-20.
- Reveal protein bands using an Odyssey imaging system (LI-COR Biosciences).

Experimental Notes:	The human MR1 antibody ABIN1537116, but not the isotype control, immunoprecipitates with human MR1 overexpressed by HEK293 cells.

Image for Validation report #102828



Validation image no. 1 for anti-Major Histocompatibility Complex, Class I-Related (MR1) (AA 312-341), (C-Term) antibody (ABIN1537116)

Lysates from human MR1-expressing HEK293 cells were immunoprecipitated by antibodies specific for MR1 (ABIN2665876, ABIN516526, ABIN1537116) or the respective isotype controls (mouse IgG2a, mouse, IgG1, rabbit IgG). Immunoprecipitants were resolved by SDS-PAGE gel followed by Western blotting analysis using MR1 antibody ABIN1537116.

Validation report #102829 for Western Blotting (WB)

Successfully validated (Western Blotting (WB))

by Dr. Randy Brutkiewicz Laboratory, Department of Microbiology and Immunology, Indiana University School of Medicine

Report Number: 102829

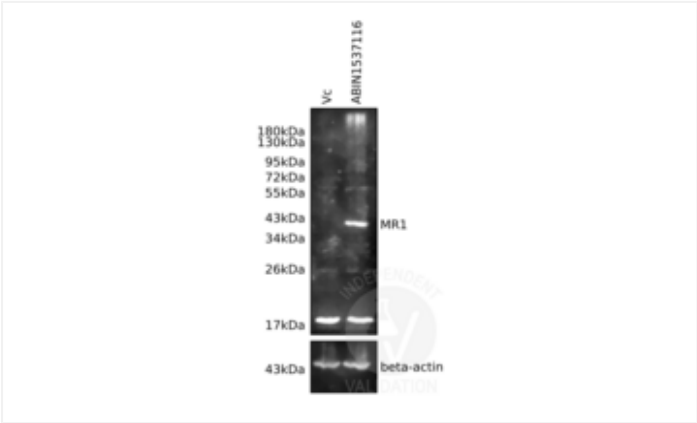
Date: Feb 20 2018

Target:	MR1
Lot Number:	SA111213CH
Method validated:	Western Blotting (WB)
Positive Control:	HEK293 cells transfected with human MR1 cDNA
Negative Control:	HEK293 cells transfected with plasmid vector only
Notes:	Passed. ABIN1537116 recognizes human MR1 overexpressed by HEK293 cells in a western blot.
Primary Antibody:	ABIN1537116
Secondary Antibody:	goat anti-rabbit Dye-IR800 conjugated antibody (Advansta, R-05060-250, lot 17083179)
Protocol:	• Grow HEK293 cells in DMEM medium (Lonza, 12-614F, lot 0000618582) supplemented with serum (Hyclone, SH30071.03, lot AAG205460) and antibiotics (Hyclone, SV30010, lot J150013), at 37°C and 5% CO_2 dish to 70-90% confluency. • Transfect cells with pCDNA 3.1 neo (-) (Invitrogen) containing human MR1 cDNA (Genecopoeia) using Polyethylenimine (Polysciences, 23966) following the manufacturer´s instructions. • Lyse cells in cold lysis buffer (10mM Tris pH7.4, 150mM NaCl, 0.5mM EDTA, 2% CHAPS). • Determine total protein content of the lysates using Commassie Protein Assay Reagent (Thermo Scientific, 1856209, lot NL179252). • Denature 200µg total protein for 5min at 95°C in 20µl Laemmli SDS sample buffer and subsequently separate them on a SDS-PAGE gel using Acrylamide/Bis Premixed (Bio-Rad, 61-0125, lot 260000477) for 2-3h at 100V. • Transfer proteins onto PVDF membrane (Millipore, IPVH00010, lot K5AA6843U) with a Western blotting system for ON at 4°C at 150mA. • Block the membrane with blocking buffer (2% BSA/PBS/0.05%Tween-20) for 1h at RT. • Incubate membrane with: primary rabbit anti-MR1 antibody (antibodies-online, ABIN1537116, lot SA111213CH) diluted 1:1000 in blocking buffer ON at 4°C. ○ loading control rabbit anti beta-actin (antibodies-online, ABIN962807) diluted 1:500 in blocking buffer ON at 4°C. • Wash membrane 3x for 10min with PBS/0.05%Tween-20.

- Incubate membrane with secondary goat anti-rabbit Dye-IR800 conjugated antibody (Advansta, R-05060-250, lot 17083179) diluted 1:10000 in PBS/0.05% Tween-20 for 1h at RT.
- Wash membrane 3x for 10min with PBS/0.05% Tween-20.
- Reveal protein bands using an Odyssey imaging system (LI-COR Biosciences).

Experimental Notes:	The human MR1 antibody ABIN1537116 reveals a protein of the expected molecular weight of MR1 in lysates of human MR1-expressing HEK293 cells. The protein bands is only visible in the positive but not the negative controls.

Image for Validation report #102829



Validation image no. 1 for anti-Major Histocompatibility Complex, Class I-Related (MR1) (AA 312-341), (C-Term) antibody (ABIN1537116)

Lysates from human MR1-expressing HEK293 cells (MR1) and vector control cells (Vc) were resolved on a 10% SDS-PAGE gel for Western blotting analysis using antibodies specific for MR1 (ABIN1537116, upper panel) and beta-actin (ABIN962807, lower panel).