

Datasheet for ABIN516526  
**anti-MR1 antibody (AA 201-300)**[Go to Product page](#)

## 3 Validations

## 2 Images

## Overview

Quantity:	100 µg
Target:	MR1
Binding Specificity:	AA 201-300
Reactivity:	Human
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This MR1 antibody is un-conjugated
Application:	ELISA, Western Blotting (WB), Immunofluorescence (IF)

## Product Details

Purpose:	Mouse monoclonal antibody raised against a partial recombinant MR1.
Immunogen:	MR1 (NP_001522, 201 a.a. ~ 300 a.a) partial recombinant protein with GST tag. MW of the GST tag alone is 26 KDa.
Sequence:	TEPPLVRVNR KETFPGVTL FCKAHGFYPP EIYMTWMKNG EEIVQEIDYG DILPSGDGTY QAWASIELDP QSSNLYSCHV EHCgvHMLVQ VPQESETIPL
Clone:	5B5
Isotype:	IgG1
Cross-Reactivity:	Human
Characteristics:	Antibody Reactive Against Recombinant Protein.

### Target Details

Target:	MR1
Alternative Name:	MR1 ( <a href="#">MR1 Products</a> )
Background:	Full Gene Name: major histocompatibility complex, class I-related Synonyms: HLALS
Gene ID:	3140
NCBI Accession:	<a href="#">NM_001531</a>
Pathways:	<a href="#">Regulation of Leukocyte Mediated Immunity</a> , <a href="#">Positive Regulation of Immune Effector Process</a> , <a href="#">Production of Molecular Mediator of Immune Response</a> , <a href="#">Cancer Immune Checkpoints</a>

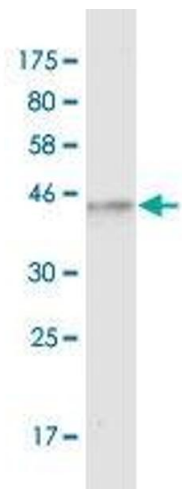
### Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Restrictions:	For Research Use only

### Handling

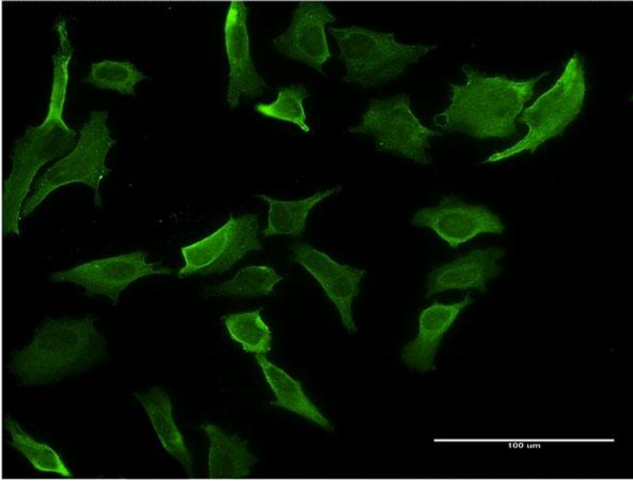
Buffer:	In 1x PBS, pH 7.4
Handling Advice:	Aliquot to avoid repeated freezing and thawing.
Storage:	-20 °C
Storage Comment:	Store at -20°C or lower. Aliquot to avoid repeated freezing and thawing.

### Validation report #102827 for Flow Cytometry (FACS)



**Western Blotting**

**Image 1.** Western Blot detection against Immunogen (36.74 KDa) .



#### Immunofluorescence

**Image 2.** Immunofluorescence of monoclonal antibody to MR1 on HeLa cell. [antibody concentration 10 ug/ml]



## Successfully validated (Immunocytochemistry (ICC))

by [Dr. Randy Brutkiewicz Laboratory, Department of Microbiology and Immunology, Indiana University School of Medicine](#)

Report Number: 101752

Date: Feb 20 2018

Target:	MR1
Lot Number:	12045-5B5
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 ABIN516526 specifically labels the targeted antigen in HEK293 ectopically expressing human MR1 in ICC.
Primary Antibody:	ABIN516526
Secondary Antibody:	FITC-conjugated donkey anti-mouse immunoglobulin antiserum (Jackson ImmunoResearch, 715-096-151, lot 76750)
Protocol:	<ul style="list-style-type: none"> <li>• 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<sub>2</sub> dish to 70-90% confluency.</li> <li>• Transfect cells with pCDNA 3.1 neo (-) (Invitrogen) containing human MR1 cDNA (Genecopoeia) using Polyethylenimine (Polysciences, 23966) following the manufacturer's instructions.</li> <li>• Plate human MR1-expressing HEK293 cells in sterile glass-bottom 35mm dishes coated with collagen (MatTek, P35GCol-1.5-14-C) to 50-80% confluency.</li> <li>• Wash cells PBS.</li> <li>• Fix cells with 4% Paraformaldehyde for 15min at RT.</li> <li>• Block cells with blocking buffer (1x PBS, 5% donkey serum, 0.3% Triton X-100) for 1h at RT.</li> <li>• Incubate cells with primary mouse anti-MR1 antibody (antibodies-online, ABIN516526, lot 12045-5B5) diluted 1:50 in dilution buffer (1x PBS, 1% BSA, 0.3% Triton X-100) and incubated ON at 4°C.</li> <li>• Wash cells 3x with PBS.</li> <li>• Incubated with secondary FITC-conjugated donkey anti-mouse immunoglobulin antiserum (Jackson ImmunoResearch, 715-096-151, lot 76750) diluted 1:50 in dilution buffer for 1h at RT.</li> <li>• Wash cells 3x with PBS.</li> <li>• To stain the nucleus, immerse cells in PBS containing Hoechst (1:2000) for 5min.</li> </ul>

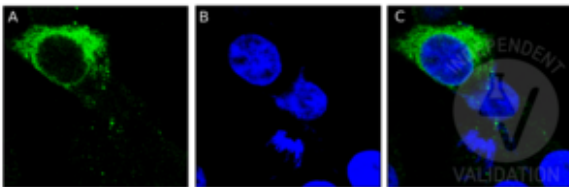
- Just prior to confocal analysis, place cells in mounting medium (10mM Tris pH8.5, 2% DABCO).
- Image cells on an Olympus 2 confocal/two-photon microscope imaging system using an oil immersion lens at 60x.

Experimental Notes: Staining with ABIN516526 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.

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## Image for Validation report #101752

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### Validation image no. 1 for anti-Major Histocompatibility Complex, Class I-Related (MR1) (AA 201-300) antibody (ABIN516526)

Human MR1-expressing HEK293 cells were stained with MR1 antibody ABIN516526 and a FITC-conjugated secondary antibody (green, A). For nuclear staining, cells were stained with Hoechst (blue, B). C shows both channels merged.



## Successfully validated (Immunoprecipitation (IP))

by [Dr. Randy Brutkiewicz Laboratory, Department of Microbiology and Immunology, Indiana University School of Medicine](#)

Report Number: 102826

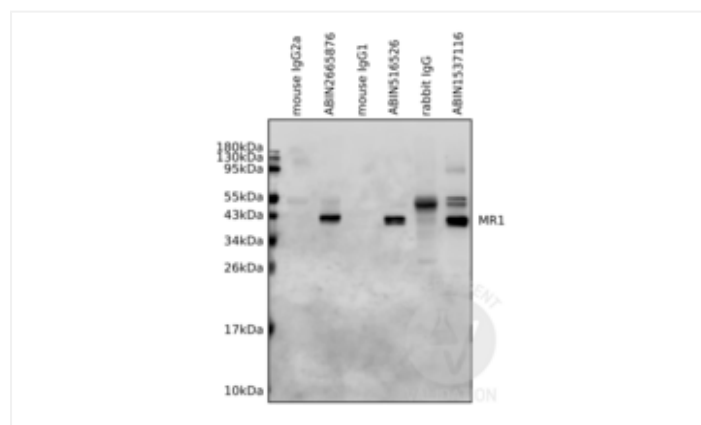
Date: Feb 20 2018

Target:	MR1
Lot Number:	12045-5B5
Method validated:	Immunoprecipitation (IP)
Positive Control:	HEK293 cells transfected with human MR1 cDNA
Negative Control:	HEK293 cells transfected with plasmid vector only
Notes:	Passed. ABIN516526 immunoprecipitates human MR1 overexpressed by HEK293 cells.
Primary Antibody:	ABIN516526
Secondary Antibody:	goat anti-rabbit Dye-IR800 conjugated antibody (Advansta, R-05060-250, lot 17083179)
Protocol:	<ul style="list-style-type: none"> <li>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<sub>2</sub> dish to 70-90% confluency.</li> <li>Transfect cells with pCDNA 3.1 neo (-) (Invitrogen) containing human MR1 cDNA (Genecopoeia) using Polyethylenimine (Polysciences, 23966) following the manufacturer's instructions.</li> <li>Lyse cells in cold lysis buffer (10mM Tris pH7.4, 150mM NaCl, 0.5mM EDTA, 2% CHAPS).</li> <li>Determine total protein content of the lysates using Commassie Protein Assay Reagent (Thermo Scientific, 1856209, lot NL179252).</li> <li>Immobilize 100µl of protein G-conjugated Sepharose beads (Pierce, product 20399, lot RI239318) ON at 4°C with <ul style="list-style-type: none"> <li>2.5µg mouse anti-MR1 antibody (antibodies-online, ABIN2665876, lot B177559),</li> <li>2.5µg mouse anti-MR1 antibody (antibodies-online, ABIN516526, lot 12045-5B5),</li> <li>2.5µg rabbit anti-MR1 antibody (antibodies-online, ABIN1537116, lot SA111213CH),</li> <li>2.5µg mouse IgG2a antibody (Biolegend, 400202, lot B153642),</li> <li>2.5µg mouse IgG1 antibody (BD, 555746, lot 3221830), or</li> <li>2.5µg rabbit IgG antibody (Santa Cruz Biotechnology, SC-5560, lot E0609).</li> </ul> </li> <li>Incubate 500µg of the cell lysates with 2.5µg of antibody-bead conjugate ON at 4°C.</li> <li>Wash lysates 4x with PBS.</li> <li>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.</li> </ul>

- 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 ABIN516526, but not the isotype control, immunoprecipitates with human MR1 overexpressed by HEK293 cells.

## Image for Validation report #102826



### Validation image no. 1 for anti-Major Histocompatibility Complex, Class I-Related (MR1) (AA 201-300) antibody (ABIN516526)

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.



## Successfully validated (Flow Cytometry (FACS))

by [Dr. Randy Brutkiewicz Laboratory, Department of Microbiology and Immunology, Indiana University School of Medicine](#)

Report Number: 102827

Date: Feb 20 2018

Target:	MR1
Lot Number:	12045-5B5
Method validated:	Flow Cytometry (FACS)
Positive Control:	HEK293 cells transfected with human MR1 cDNA
Negative Control:	HEK293 cells transfected with plasmid vector only
Notes:	Passed. ABIN2665876 recognizes human MR1 overexpressed by HEK293 cells and can be used in flow cytometry.
Primary Antibody:	ABIN516526
Secondary Antibody:	PE-conjugated rabbit anti-mouse immunoglobulin antiserum (Jackson ImmunoResearch, 115-116-146, lot 120701)
Protocol:	<ul style="list-style-type: none"> <li>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<sub>2</sub> dish to 70-90% confluency.</li> <li>Transfect cells with pCDNA 3.1 neo (-) (Invitrogen) containing human MR1 cDNA (Genecopoeia) using Polyethylenimine (Polysciences, 23966) following the manufacturer's instructions.</li> <li>Surface staining: <ul style="list-style-type: none"> <li>Wash 0.5x10<sup>6</sup> cells 3x with HBSS/0.1% BSA.</li> <li>Incubate cells with mouse anti-MR1 antibody (antibodies-online, ABIN516526, lot 12045-5B5) diluted 1:100 or mouse anti-MR1 antibody (antibodies-online, ABIN2665876, lot B177559) diluted 1:100 for 30min on ice.</li> <li>Wash cells 3x with HBSS/0.1% BSA.</li> <li>Incubate cells with a PE-conjugated rabbit anti-mouse immunoglobulin antiserum (Jackson ImmunoResearch, 115-116-146, lot 120701) diluted 1:100 for 30min on ice.</li> </ul> </li> <li>Total cell staining: <ul style="list-style-type: none"> <li>Fix 0.5x10<sup>6</sup> cells in 1% paraformaldehyde for 10min at RT.</li> <li>Wash cells with HBSS/BSA.</li> <li>Permeabilize cells in HBSS/BSA with 0.1% saponin for 10min at RT.</li> <li>Incubate cells with mouse anti-MR1 antibody (antibodies-online, ABIN516526, lot 12045-</li> </ul> </li> </ul>

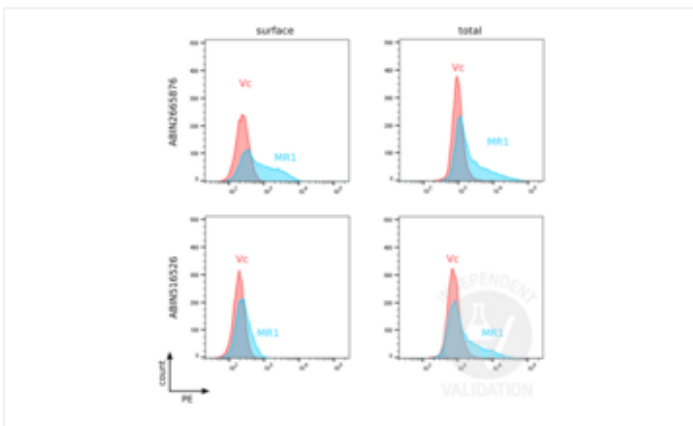


5B5) diluted 1:100 or mouse anti-MR1 antibody (antibodies-online, ABIN2665876, lot B177559) dilute 1:100 for 30min at RT in the presence of 0.1% saponin.

- Wash cells 3x with HBSS/0.1% BSA/0.1% saponin.
- Incubate cells with PE-conjugated rabbit anti-mouse immunoglobulin antiserum (Jackson ImmunoResearch, 115-116-146, lot 120701) diluted 1:100 for 30min at RT.
- Acquire flow cytometry data using a flow cytometry using LSR 4 (BD Biosciences) followed data analysis using FlowJo software.

Experimental Notes: The human MR1 antibody ABIN2665876 shows specific staining for human MR1.

Image for Validation report #102827



**Validation image no. 1 for anti-Major Histocompatibility Complex, Class I-Related (MR1) (AA 201-300) antibody (ABIN516526)**

Human MR1-expressing HEK293 cells (MR1) and vector control cells (Vc) were stained with MR1 antibodies ABIN516526 or ABIN2665876 followed by PE-conjugated anti-mouse secondary antibody. For total MR1 staining, cells were permeabilized with 0.1% saponin and stained with primary and secondary antibodies. Cells were analyzed by flow cytometry.