PRODUCT INFORMATION

MR1 Immunoaffinity Sorbent

Item No. 38215

Overview and Properties

Contents: This vial contains 400 µl of CNBr-activated Sepharose 4B coupled to a human MR1 Monoclonal Antibody (Clone 2H7) (Item No. 34257). The product is supplied as a 50% slurry in PBS, pH 7.2, with 0.02% sodium azide.

Synonyms: Class I Histocompatibility Antigen-like Protein, Major Histocompatibility Complex Class I-related Gene Protein, MHC Class I-related Gene Protein, MR1 Immunoaffinity Beads, MR1 Immunoaffinity Resins

Immunogen: Recombinant human MR1 (aa 22-292) protein

Cross Reactivity: (+) MR1

Species Reactivity: (+) Human; other species not tested

Uniprot No.: Q95460

Form: Liquid

Storage: 4°C (as supplied)

Stability: ≥1 year

Storage Buffer: PBS, pH 7.2, with 0.02% sodium azide

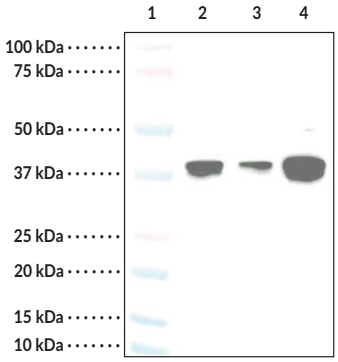
Clone: 2H7

Host: Mouse

Isotype: IgG1

Applications: Suitable for immunoprecipitation (IP) (LC/MS certified) and Western blot (WB), working concentration/dilution should be determined empirically.

Images



Lane 1: MW Markers
Lane 2: Lysate Input (10 µg)
Lane 3: Flow Through (10 µg)
Lane 4: IP Eluate

WB of MR1 Immunoaffinity Sorbent. MR1 overexpressing cells were lysed and the lysate applied to the MR1 immunoaffinity sorbent with overnight incubation. The sorbent was washed and eluted with SDS-PAGE sample buffer followed by WB analysis using human MR1 Monoclonal Antibody (Clone 2H7) (Item No. 34257).

Identified Proteins (1694)	Accession Number	Molecular Weight	64649 SpC	64650 SpC
Tubulin alpha-1B chain OS=Homo sapiens OX=9606 GN=TUBA1B PE=1 SV=1	sp|P68363	50 kDa	497	87
Tubulin beta chain OS=Homo sapiens OX=9606 GN=TUBB PE=1 SV=2	sp|P07437	50 kDa	413	158
Tubulin beta-4B chain OS=Homo sapiens OX=9606 GN=TUBB4B PE=1 SV=1	sp|P68371	50 kDa	377	142
Tubulin beta-2B chain OS=Homo sapiens OX=9606 GN=TUBB2B PE=1 SV=1	sp|Q9BVA	50 kDa	373	146
Tubulin beta-2A chain OS=Homo sapiens OX=9606 GN=TUBB2A PE=1 SV=1	sp|Q13885	50 kDa	365	145
Tubulin alpha-4A chain OS=Homo sapiens OX=9606 GN=TUBA4A PE=1 SV=1	sp|P68366	50 kDa	395	0
Tubulin alpha-1C chain OS=Homo sapiens OX=9606 GN=TUBA1C PE=1 SV=1	sp|Q9BQE	50 kDa	362	83
Tubulin beta-4A chain OS=Homo sapiens OX=9606 GN=TUBB4A PE=1 SV=2	sp|P04350	50 kDa	288	96
Major histocompatibility complex class I-related gene protein OS=Homo sapier	sp|Q9546	39 kDa	181	169
Heat shock 70 kDa protein 1A OS=Homo sapiens OX=9606 GN=HSPA1A PE=1 SV=1	sp|P0DMV	70 kDa	189	147

Immunoprecipitation and analysis by LC-MS/MS. IP eluates were separated on Bis-Tris mini-gels, and Coomassie stained followed by excision. The bands were trypsin digested, and analyzed by LC-MS/MS by HPLC using trapping and analytical columns with C18 resin and MS and MS/MS using a ThermoFisher Fusion Lumos Orbitrap. Analysis was done using Scaffold Proteomics Software.

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Laboratory Procedures

The MR1 Immunoaffinity Sorbent is designed for the immunoprecipitation of MR1 from biological samples. The immunoprecipitated material can be eluted and analyzed by WB using an MR1 polyclonal antibody or by LC-MS/MS proteomic analysis.

A typical procedure for immunoprecipitation is provided as follows:

1. Resuspend the sorbent by gentle flicking or pipetting (*do not vortex the sorbent at any point*).
2. Wash 40-100 µl (20-50 µl of bead volume) of the MR1 Immunoaffinity Sorbent using 1 ml of the intended buffer that will be used for sample lysis or PBS-T (0.1% Tween). Spin down at 500 x g for five minutes and discard the supernatant.
3. Repeat step (2) two additional times.
4. Add 40-100 µl of MR1 Immunoaffinity Sorbent (20-50 µl bead volume) to cell/tissue lysate containing 0.5-5 mg of total protein in a 1.5 ml tube.
5. Incubate the sorbent containing sample overnight at 4°C with inversion.
6. Centrifuge the beads at 500 x g for five minutes. Collect and save the supernatant for depletion analysis.
7. Wash beads with 1 ml of PBS-T (0.1% Tween) and spin down at 500 x g for five minutes discarding the supernatant.
8. Repeat step (7) two additional times. Carefully remove as much supernatant as possible without disturbing the beads following the last wash.
9. *WB analysis*: Resuspend the beads in an equal volume of Laemmili sample buffer and boil for five minutes. The sample is now ready for SDS-PAGE and subsequent WB analysis.
   LC/MS analysis: Complete three additional washes with water to remove buffer salts/detergent. Resuspend the beads in 0.1% trifluoroacetic acid in water and incubate for five minutes at room temperature. The sample is now ready for LC/MS analysis.

Description

Major histocompatibility complex (MHC) class I-related gene protein (MR1) is a non-polymorphic MHC class Ib antigen-presenting cell surface molecule that is required for T cell receptor-mediated activation of mucosal-associated invariant T (MAIT) cells.[1,2] It is composed of α1 and α2 domains, which form an antigen-binding pocket, and an α3 domain that interacts with β2-microglobulin.[2] *MR1* mRNA is ubiquitously expressed and, following translation, MR1 protein is localized to the endoplasmic reticulum in a partially folded state. Upon binding of a riboflavin-derived microbial antigen, MR1 undergoes a conformational change and translocates to the cell surface where it induces MAIT cell activation *via* an interaction with the MAIT cell T cell receptor and activates various immunomodulatory effects, including cytokine release, initiation of adaptive immune responses, and promotion of tissue repair.[3] Cayman's MR1 Immunoaffinity Sorbent is designed for immunoprecipitation (IP) of MR1 protein from biological samples. This is an effective way to concentrate MR1 protein for subsequent detection by a different MR1 antibody by immunoprecipitation (IP) (LC/MS certified) and Western blot (WB). The MR1 affinity sorbent consists of Cayman's MR1 Monoclonal Antibody (Clone 2H7) (Item No. 34257) coupled to CNBr-activated Sepharose 4B.

References

1. Lamichhane, R. and Ussher, J.E. Expression and trafficking of MR1. *Immunology* **151(3)**, 270-279 (2017).
2. Krovi, S.H. and Gapin, L. Structure and function of the non-classical major histocompatibility complex molecule MR1. *Immunogenetics* **68(8)**, 549-559 (2016).
3. McWilliam, H.E.G. and Salio, M. Understanding and modulating the MR1 metabolite antigen presentation pathway. *Mol. Immunol.* **129**, 121-126 (2021).

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