Extracellular Vesicle Human CD9/CD63/CD81 Antibody Panel

Antibody panel for the detection of extracellular vesicles using CD9, CD63, and CD81 markers

Catalog #100-0211 1 Kit

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Product Description

The Extracellular Vesicle Human CD9/CD63/CD81 Antibody Panel is suitable for the detection of extracellular vesicles (EVs) derived from human cells. It comprises three primary antibodies that are immunoreactive toward human CD9, CD63, and CD81; these are proteins that are typically expressed on EVs and widely used as markers to analyze and isolate these cell-derived particles. CD9, CD63, and CD81 belong to the tetraspanin family of membrane proteins, which possess four transmembrane domains and interact with diverse proteins on the cell surface to form multimolecular networks termed tetraspanin-enriched microdomains. CD9, CD63, and CD81 proteins are expressed on the surface of many cells, including B cells, T cells, NK cells, monocytes, dendritic cells, thymocytes, endothelial cells, and fibroblasts, and are involved in modulating a variety of cellular processes including cell activation, adhesion, differentiation, and tumor invasion. The antibodies provided in this panel have been reported for use in analyzing primary cells, cell lines, and EVs by ELISA, flow cytometry, immunocytochemistry, immunoprecipitation, and Western blotting. They have been reported to cross-react with their cognate antigens in non-human primates, including baboons and rhesus and cynomolgus macaques.

Product Information

The following products comprise the Extracellular Vesicle Human CD9/CD63/CD81 Antibody Panel and are also available for individual sale. For additional information, including storage instructions, refer to the applicable Product Information Sheet (PIS) as indicated in the table below.

PRODUCT NAME	CATALOG #	QUANTITY	UNIT SIZE	PIS DOCUMENT #
Anti-Human CD9 Antibody, Clone HI9a	100-0138	1	100 µg	10000006614
Anti-Human CD63 Antibody, Clone H5C6	100-0139	1	100 µg	10000006615
Anti-Human CD81 Antibody (TAPA-1), Clone 5A6	100-0209	1	100 µg	10000006895

Materials Required But Not Included

An anti-mouse IgG secondary antibody conjugated to an appropriate label is required for detection. For Western blotting, we recommend using the following horseradish peroxidase (HRP)-conjugated anti-mouse IgG secondary antibodies:

- Peroxidase AffiniPure Goat Anti-Mouse IgG (H+L) (Jackson ImmunoResearch Laboratories, Inc., Catalog #115-035-062) or
- Goat anti-Mouse IgG (H&L), F(ab')2 fragment, HRP Conjugate, Cross-Adsorbed (Tonbo Biosciences, Catalog #72-8096-U500).

Alternatively, a monoclonal or polyclonal HRP-conjugated anti-mouse IgG1 secondary antibody may be used.

Directions for Use

The following protocol is recommended for Western blotting.

1. Electrophorese the isolated EV samples in a non-reducing gel and transfer to a polyvinylidene difluoride (PVDF) membrane.
2. Incubate the PVDF membrane in blocking solution (phosphate-buffered saline [PBS] + 5% bovine serum albumin [BSA] + 0.2% TWEEN® 20) at room temperature (15 - 25°C) for 1 hour with gentle agitation.
3. Dilute primary antibodies (CD9, CD63, and CD81) in blocking solution. Prepare a volume sufficient to immerse the membrane. See Table 1 for recommended working concentrations. For the CD63 antibody, refer to the lot-specific concentration on the Certificate of Analysis (CoA).
   NOTE: The expected molecular masses of the target markers recognized by these antibodies are ~ 24 kDa (CD9), 30 - 60 kDa (CD63), and ~ 26 kDa (CD81). Therefore, it is not recommended to incubate the membrane with the anti-CD9 and anti-CD81 antibodies together, as it may be difficult to distinguish separate bands for CD9 and CD81.
4. Decant the blocking solution from the membrane. Add diluted primary antibodies (CD9, CD63, and CD81) to the membrane and incubate at room temperature for 30 minutes with gentle agitation, then at 2 - 8°C overnight without agitation.

Table 1. Recommended Working Concentrations of Primary Antibodies

PRIMARY ANTIGEN	TARGET ANTIGEN	HOST SPECIES	ISOTYPE	WORKING CONCENTRATION*
Anti-Human CD9 Antibody, Clone HI9a	CD9	Mouse	IgG1, kappa	0.5 - 1 µg/mL
Anti-Human CD63 Antibody, Clone H5C6	CD63	Mouse	IgG1, kappa	1 - 2 µg/mL
Anti-Human CD81 Antibody (TAPA-1), Clone 5A6	CD81	Mouse	IgG1, kappa	1 - 2 µg/mL

*Titrate each primary antibody for optimal performance.

5. Decant the primary antibody solution. Rinse the membrane with wash buffer (PBS + 0.2% TWEEN® 20) 4 x 5 minutes at room temperature with gentle agitation.
6. Dilute the secondary antibody (e.g., goat anti-mouse IgG antibody, HRP-conjugated) in blocking solution. Prepare a volume sufficient to immerse the membrane. A suggested dilution for the secondary antibody is 1 in 5000, though titration of the antibody may be required for optimal performance. Use the manufacturer's recommendation, if provided.
7. After the final rinse, decant the wash buffer and add the diluted secondary antibody to the membrane. Incubate at room temperature for 1 hour with gentle agitation.
8. Decant the secondary antibody solution and rinse the membrane with wash buffer 4 x 5 minutes at room temperature with gentle agitation.
9. Develop the membrane using a substrate appropriate for HRP according to the manufacturer's instructions.

References

1. Bachurski D et al. (2019) Extracellular vesicle measurements with nanoparticle tracking analysis – An accuracy and repeatability comparison between NanoSight NS300 and ZetaView. J Extracell Vesicles 8(1): 1596016. (FC, IF, WB)
2. Susa KJ et al. (2019) A dynamic interaction between CD19 and the tetraspanin CD81 controls B cell co-receptor trafficking. bioRxiv. Epub ahead of print, DOI: 10.1101/768275. (Epitope-mapping, FC, Immunoaffinity chromatography, IP)
3. Earnest JT et al. (2017) The tetraspanin CD9 facilitates MERS-coronavirus entry by scaffolding host cell receptors and proteases. PLoS Pathog 13(7): e1006546. (ICC, IF, WB)
4. Oliveira-Rodríguez M. (2016) Development of a rapid lateral flow immunoassay test for detection of exosomes previously enriched from cell culture medium and body fluids. J Extracell Vesicles 5: 10.3402/31803. (ELISA)
5. Rappa G et al. (2015) Tetraspanin CD9 determines invasiveness and tumorigenicity of human breast cancer cells. Oncotarget 6(10): 7970–91. (FACS, FC, ICC, IF, WB)
6. Shi M. (2014) Plasma exosomal α-synuclein is likely CNS-derived and increased in Parkinson's disease. Acta Neuropathol 128(5): 639–50. (IP)

Abbreviations: CellSep: Cell separation; ChIP: Chromatin immunoprecipitation; FA: Functional assay; FACS: Fluorescence-activated cell sorting; FC: Flow cytometry; ICC: Immunocytochemistry; IF: Immunofluorescence microscopy; IHC: Immunohistochemistry; IP: Immunoprecipitation; RIA: Radioimmunoassay; WB: Western blotting