

Chapter 5

Antibodies for EV research

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Antibodies for EV research

Introduction

Antibodies are an essential tool for basic research, development of diagnostic tests and therapeutics for human disease. Extracellular vesicles (EVs), including exosomes, express antigens with 3D conformations and/or post-translational modifications that often differ from the cellular counterpart. For this reason, most of the antibodies currently available on the market fail to recognize exosome-associated antigens with sufficient sensitivity and specificity. HBM-LS has validated a list of polyclonal and monoclonal antibodies against common (CD63, CD81, ALIX) and disease-specific (cancer and neurodegenerative diseases) exosomal markers.

Antibodies for common and disease-associated EV markers

Cat. Code	Antibody	Type	Applications
Antibodies for common EV Markers			
HBM-TSG101-####	Anti human TSG101	Mouse monoclonal unconjugated	WB, IHC
HBM-CD9-####	Anti human CD9	Mouse monoclonal unconjugated	WB, FACS, ELISA, IP, IHC
HBM-CD9B-####	Anti human CD9	Mouse monoclonal biotin conjugated	WB,, ELISA, IP
HBM-CD63-####	Anti human CD63	Mouse monoclonal unconjugated	WB, FACS, ELISA, IP, IHC
HBM-CD41-EM1-####	Anti human CD41	Mouse monoclonal unconjugated	FACS, ELISA
HBM-CD81-EM4-####	Anti human CD81	Mouse monoclonal unconjugated	WB, FACS, ELISA, IP, IHC
HBM-ALIX-####	Anti human Alix	Mouse monoclonal unconjugated	WB, FACS, IF
HBM-FLOT-####	Anti human Flotillin	Rabbit polyclonal unconjugated	WB,FACS, ELISA
HBM-RAB5-PR1-####	Anti human RAB5	Rabbit polyclonal unconjugated	WB, FACS, ELISA, IP
HBM-CD9M-####	Anti mouse CD9	Mouse monoclonal unconjugated	WB, FACS, ELISA, IP
Antibodies for EV-associated disease markers			
HBM-HSP70-SR1-####	Anti human HSP70	Rabbit polyclonal unconjugated	WB, FACS, ELISA
HBM-SF4-PR2-####	Anti human TM9SF4	Rabbit polyclonal unconjugated	WB, IP
HBM-CD44-EM1-####	Anti human CD44	Mouse monoclonal unconjugated	WB, ELISA, IP
HBM-CAV1-D4-####	Anti human Caveolin 1	Mouse monoclonal unconjugated	WB, FACS

List of abbreviations:

ELISA: Enzyme-Linked Immunosorbent Assay

FACS: Fluorescent-Activated Cell Sorting

IHC: Immunohistochemistry

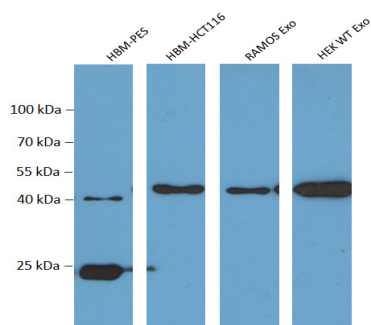
IP: Immunoprecipitation

IF: Immunofluorescence

WB: Western Blotting

Anti human TSG101 antibody

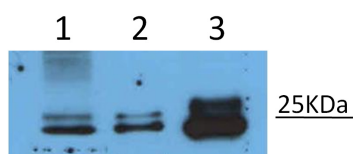
Description	TSG101, a 46 kDa protein, is the product of a recently identified Tumor Susceptibility Gene whose inactivation in mouse fibroblasts results in cell transformation and the ability of those cells to form tumors in nude mice. TSG101 is highly expressed internally in EVs and it is considered one of the common markers for exosome detection.
Cat Num/Amount	HBM-TSG101-100 (100 µg)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	WB, IHC



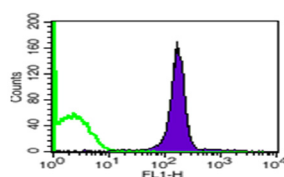
Western Blotting
Detection of TSG101 in 20 µg of Exosome Standards from:
1-HBM-PES: healthy human serum
2-HBM-HCT116: HCT116 cell culture medium
3-RAMOS Exo: exosomes purified from RAMOS cell medium
4- HEK293 WT Exo: exosomes purified from HEK293 wild type cell medium.

Anti human CD9 antibody

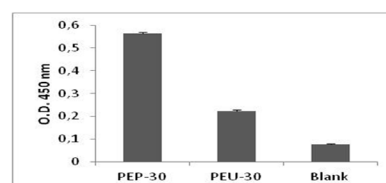
Description	Anti-CD9 recognizes a human 24-kiloDalton (kDa) single-chain cell-surface glycoprotein (p24) belonging to the tetraspanin family. CD9 has a very broad tissue distribution and is abundant on exosome membranes. HBM-LS offers monoclonal anti-CD9 antibodies, unconjugated or biotin conjugated, recognizing the specific antigen on both cell lines and extracellular vesicles.
Cat Num/Amount	HBM-CD9-100 (100 µg)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	WB, ELISA, FACS, IP, IHC
References	- Rampini, S., et al. "Micromagnet arrays for on-chip focusing, switching, and separation of superparamagnetic beads and single cells." Lab on a Chip 15.16 (2015): 3370-3379. - Gagni, Paola, et al. "Combined mass quantitation and phenotyping of intact extracellular vesicles by a microarray platform." Analytica Chimica Acta (2015).



Western Blotting
1- MM1 (melanoma cell) lysate (20 µg)
2- MM1 cells purified exosomes (20 µg)
3-Plasma healthy donors purified exosomes (20 µg)



FACS
CD9 staining of exosomes purified from human plasma



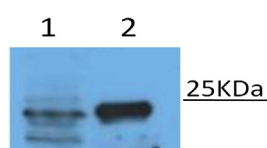
ELISA
CD9 detection in purified exosomes from human plasma (PEP) and urine (PEU), 30 µg



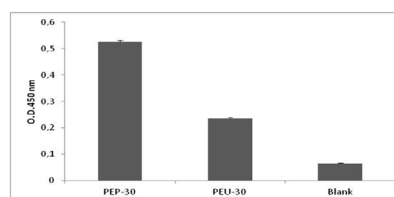
Antibodies for EV research

Anti human CD9 antibody biotin conjugated

Description	Anti-CD9 recognizes a human 24-kiloDalton (kDa) single-chain cell-surface glycoprotein (p24) belonging to the tetraspanin family. CD9 has a very broad tissue distribution and is abundant on exosome membranes. HBM offers two monoclonal anti-CD9 antibodies, unconjugated or biotin conjugated, recognizing the specific antigen on both cell lines and extracellular vesicles. HBM antibodies are compatible with ELISA, WB and flow cytometry applications.
Cat Num/Amount	HBM-CD9B-100 (100 µg)
Type	Mouse monoclonal biotin conjugated
Reactivity	Human
Applications	WB, ELISA, IP
References	<ul style="list-style-type: none"> - Rampini, S., et al. "Micromagnet arrays for on-chip focusing, switching, and separation of superparamagnetic beads and single cells." Lab on a Chip 15.16 (2015): 3370-3379. - Gagni, Paola, et al. "Combined mass quantitation and phenotyping of intact extracellular vesicles by a microarray platform." Analytica Chimica Acta (2015).



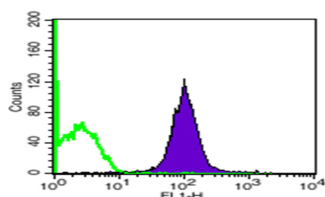
Western blotting
1- COLO1 cell lysates (20 µg)
2- CD9 detection in plasma purified exosomes (20 µg)



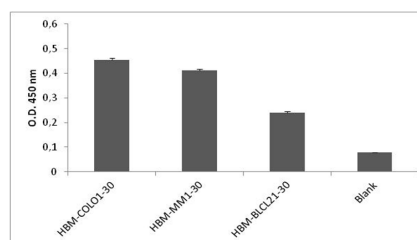
ELISA
CD9 detection in purified exosomes from human plasma (PEP) and urine (PEU), 30 µg

Anti human CD63 antibody

Description	Anti-CD63 recognizes an extracellular fragment of CD63, a 56 kiloDalton (kDa), type III lysosomal glycoprotein, belonging to the tetraspanin family. CD63 is expressed by granulocytes, platelets, T-cells, monocytes/macrophages and endothelial cells. CD63 protein is a canonical exosome marker and currently used to characterize exosome populations from a variety of body fluids.
Cat Num/Amount	HBM-CD63-100 (100 µg)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	WB, ELISA, FACS, IP, IHC



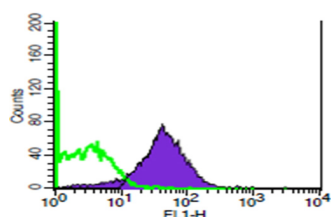
FACS
CD63 staining of exosomes purified from human plasma



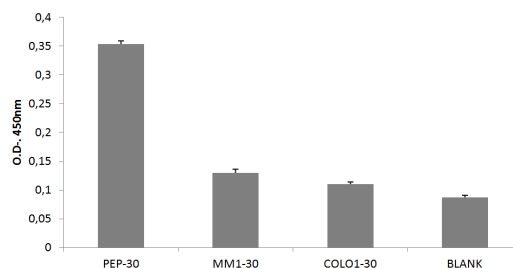
ELISA
CD63 detection in purified exosomes derived from cell supernatants (COLO1-30, MM1-30, BLCL21-30)

Anti human CD41 antibody

Description	Integrin alpha chain 2b, also known as CD41, is an heterodimeric integral membrane protein. It undergoes post-translational modifications that result in two polypeptide chains linked by a disulfide bond. CD41 is expressed on platelets and megakaryocytes, but also on early embryonic hematopoietic stem cells and related exosomes.
Cat Num/Amount	HBM-CD41-EM1-100 (100 µg)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	FACS, ELISA



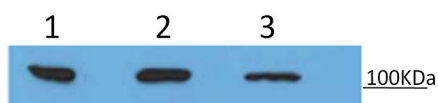
FACS
CD41 staining of exosomes purified from human plasma



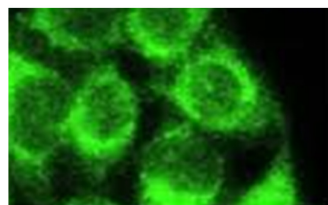
ELISA
PEP-30: 30 ug of purified exosomes from human healthy donors plasma.
MM1-30: 30 ug of purified exosomes from MM1 cell supernatant
COLO1-30: : 30 ug of purified exosomes from COLO1 cell supernatant

Anti human Alix antibody

Description	Alix protein, named also ALG2 interacting protein or PDCD6-interacting protein, is a cytoplasmic protein that interacts with apoptosis associated-proteins. Alix plays an active role in exosome biogenesis and it is a useful internal marker for the analysis of exosomal proteins with western blotting.
Cat Num/Amount	HBM-ALIX-SM1-100 (100 µg)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	WB, FACS, IF



Western blotting
1- MM1 (melanoma cell) lysate (20 ug)
2- MM1 cells purified exosomes (20 ug)
3- Plasma healthy donors purified exosomes (20 ug)



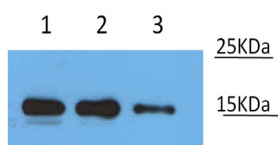
Immunofluorescence
Immunofluorescence staining of HeLa cells.



Antibodies for EV research

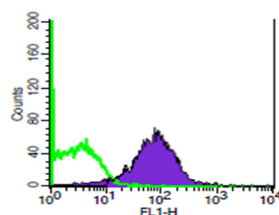
Anti human CD81 antibody

Description	CD81 (TAPA1), a member of the tetraspanin family, is virtually expressed on all nucleated cells, but in particular on germinal center B cells. CD81 forms complexes with other tetraspanin proteins, integrins and coreceptors. CD81 is expressed on exosome membranes. HBM anti-CD81 monoclonal antibody is adapt for specific antigen recognition from cell lysates and exosomes using the techniques indicated below.
Cat Num/Amount	HBM-CD81-EM1-100 (100 μ g)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	WB, ELISA, FACS, IP, IHC
References	- Gagni, Paola, et al. "Combined mass quantitation and phenotyping of intact extracellular vesicles by a microarray platform." Analytica Chimica Acta (2015).



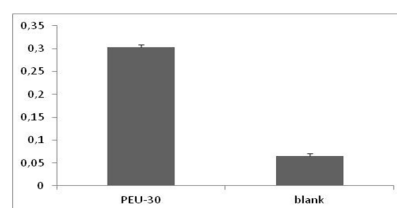
Western blotting

- 1- MM1 (melanoma cell) lysate (20 ug)
- 2- MM1 cells purified exosomes (20 ug)
- 3- Plasma healthy donors purified exo-



FACS

CD81 staining of COLO1 cell purified exosomes

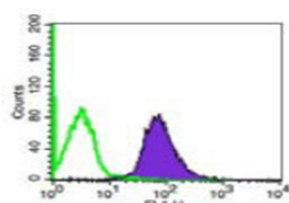


ELISA

CD81 on purified exosomes from urine (PEU), 30 ug

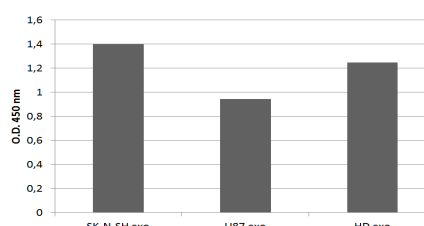
Anti human Flotillin antibody

Description	Flotillin belongs to the band 7.2/stomatin protein family and appears to be strongly expressed in muscle cells and fibroblasts. Flotillin expression is also correlated with Alzheimer development. Flotillin is highly expressed on exosomes and appears to be involved in exosome release mechanism. It is considered a common marker for exosomes analyses.
Cat Num/Amount	HBM-FLOT-SR1-100 (100 μ g)
Type	Rabbit polyclonal unconjugated
Reactivity	Human
Applications	WB, ELISA, FACS
References	Gagni, Paola, et al. "Combined mass quantitation and phenotyping of intact extracellular vesicles by a microarray platform." Analytica Chimica Acta (2015).



FACS

Purified exosomes from MM1 (30 ug) stained by anti-Flotillin antibody

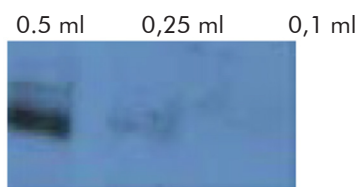


ELISA

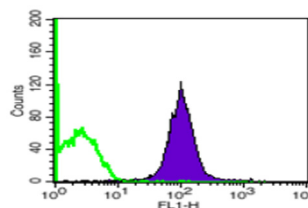
Detection of different exosomes purified from cell culture supernatants or human plasma (HD), performed with anti-Flotillin

Anti human Rab5 antibody

Description	Rab5 is a small GTPase belonging to Ras superfamily of monomeric G proteins. Rab GTPases play an essential role in the regulation of membrane traffic and are involved in vesicle formation and transport and fusion to the membrane. Rab5 is expressed on exosome membranes and it might have an active role during endo/exocytotic processes of microvesicles through the plasma membrane.
Cat Num/Amount	HBM-RAB5-PR1-100 (100 µg)
Type	Rabbit polyclonal unconjugated
Reactivity	Human
Applications	WB, ELISA, FACS, IP
References	Gagni, Paola, et al. "Combined mass quantitation and phenotyping of intact extracellular vesicles by a micro-array platform." <i>Analytica Chimica Acta</i> (2015).



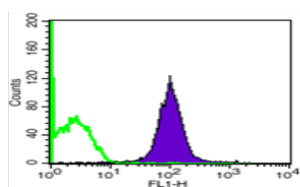
Western blotting
Exosomes purified from 0.5, 0.25, 0.1 ml of human plasma demonstrate different intensity of Rab5 expression



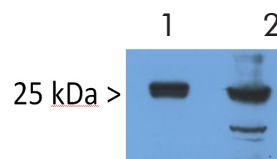
FACS
Purified exosomes from COLO1 cell line detected by Rab5 antibody

Anti mouse CD9 antibody

Description	Anti-CD9 recognizes a human 24-kiloDalton (kDa) single-chain cell-surface glycoprotein (p24) belonging to the tetraspanin family. CD9 has a very broad tissue distribution and is abundant on exosome membranes. HBM offers a monoclonal anti-CD9 antibody unconjugated reactive against mouse antigen and able to identify CD9 in mouse derived exosomes.
Cat Num/Amount	HBM-CD9M-050 (50 µg) HBM-CD9M-100 (100 µg)
Type	Mouse monoclonal unconjugated
Reactivity	Mouse
Applications	WB, ELISA, FACS, IP, IHC



FACS
CD9 staining of B16F10 mouse cell purified exosomes



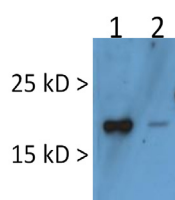
Western blotting
1- 20ug of whole lysate of B16F10 cell supernatant purified exosomes
2- 20 ug of exosomes isolated from mouse plasma



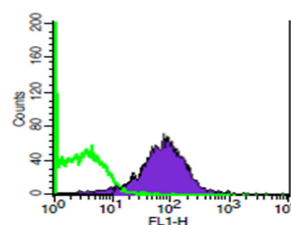
Antibodies for EV research

Anti human Caveolin antibody

Description	The lipid raft-associated protein caveolin-1 (CAV1) is the major component of the inner surface of caveolae, small invaginations of the plasma membrane. Caveolin is a transmembrane adaptor molecule that can simultaneously recognize GPI-linked proteins and interact with downstream cytoplasmic signaling molecules. It is highly expressed on exosomes derived from tumor tissue.
Cat Num/Amount	HBM-CAV1-D4-100 (100 μ g)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	WB, FACS



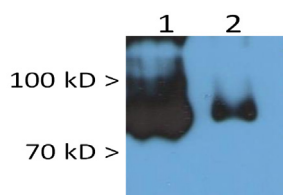
Western blotting
1- 20 ug of whole lysate of melanoma cell derived exosomes (MM1)
2- 20 ug of whole MM1 cell lysate



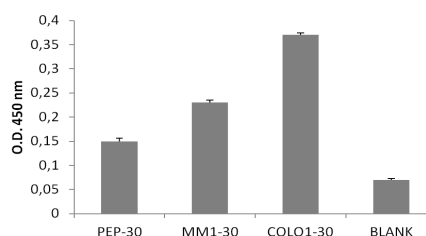
FACS
Staining of Cav1 in MM1 purified EVs

Anti human CD44 antibody

Description	CD44, known also as HCAM, is a 742 aminoacid protein involved in lymphocyte activation, homing and hematopoiesis, CD44 is expressed in multiple isoforms. CD44 is highly expressed in cancer tissues and tumor-derived exosomes, suggesting a role in tumor progression and metastasis.
Cat Num/Amount	HBM-CD44-EM1-100 (100 μ g)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	WB, ELISA, IP



Western blotting
1- 20ug of whole lysate of MM1 cell supernatant purified exosomes
2- 20 ug of purified exosomes from human plasma of healthy donors (PEP)

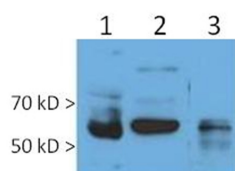


ELISA

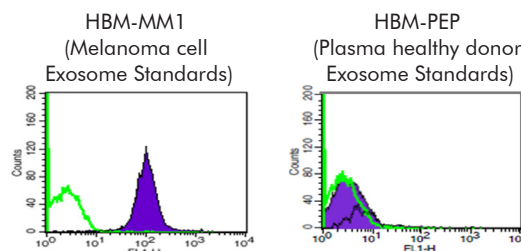
PEP-30: 30 ug of purified exosomes from human healthy donors plasma.
MM1-30: 30 ug of purified exosomes from MM1 cell supernatant
COLO1-30: : 30 ug of purified exosomes from COLO1 cell supernatant

Anti human TM9SF4 antibody

Description	TM9SF4 (TUCAP1) is a newly discovered tumor-associated protein of unknown function that belongs to the Trans-Membrane 9 Superfamily (TM9SF). These proteins are characterized by the presence of a large variable extracellular N-terminal domain followed by nine putative transmembrane domains and a conserved C-terminal domain. TM9SF4 is mainly expressed on exosomes derived from tumor tissue. HBM offers three different monoclonal antibodies that recognize the protein on both exosomes and cell lysates.
Cat Num/Amount	HBM-SF4-PMG1-100 (100 µg)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	WB, FACS, IP



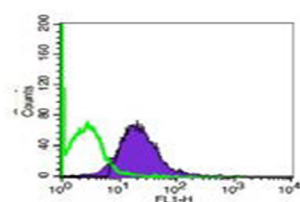
Western blotting
1- 20 ug of whole lysate of melanoma cell line
2- 20ug of whole lysate of MM1 cell supernatant purified exosomes
3- 20 ug of purified exosomes from human plasma of healthy donors (PEP)



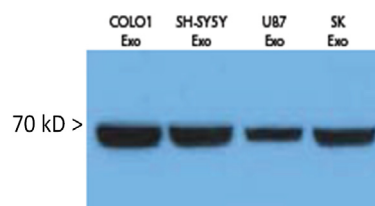
FACS
Staining of TM9SF4 on MM1 purified exosomes vs human plasma exosomes (PEP).

Anti human HSP70 antibody

Description	Heat shock protein 70 (HSP70) is a molecular chaperone that facilitates the assembly of multi-protein complexes and trafficking of polypeptides across cell membranes. HSP70 is active in promoting tumorigenesis and functions as an anti-apoptotic factor. It is highly expressed on exosomes derived from tumor tissues.
Cat Num/Amount	HBM-HSP70-SR1-100 (100 µg)
Type	Rabbit polyclonal unconjugated
Reactivity	Human
Applications	WB, ELISA, FACS, IP
References	Gagni, Paola, et al. "Combined mass quantitation and phenotyping of intact extracellular vesicles by a microarray platform." <i>Analytica Chimica Acta</i> (2015).



FACS
Staining of COLO1 derived exosomes with anti-HSP70



Western blotting
Analysis of HSP70 expression in different exosomes derived from tumoral cell lines:
1- COLO1; 2- SH-SY5Y; 3 - U87; 4 - SK

