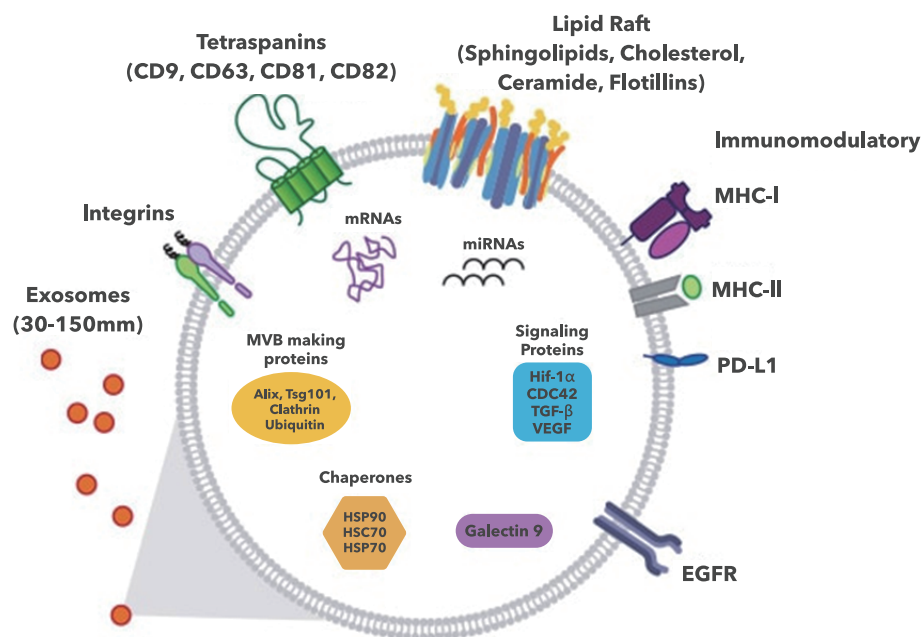


Protein Markers for Exosome Characterization

國際胞外體學會(International Society for Extracellular Vesicles, ISEV)論述了在人類樣本中進行外泌體(Exosome)研究的基礎要求。這些要求包括藉由ISEV建議的蛋白質標記區分細胞外囊泡(Extracellular Vesicles, EVs)與培養基或生物體液(如血清、血漿和尿液)中的污染成分。通過偵測至少一種跨膜或GPI錨定蛋白(Anchored protein)和一種細胞質脂質(Cytosolic Lipid)或細胞膜相關蛋白來確認外泌體的存在。為了評估細胞成分對外泌體製劑的污染程度，檢測項目還應包括與EVs幾乎沒有關聯性的目標蛋白，如白蛋白(albumin)、ApoA1、Calnexin或核糖體蛋白(Ribosomal protein)S6/RPS6。



General Exosome Markers: Tetraspanins

識別外泌體最常見的跨膜標記物來自四跨膜蛋白(Tetraspanin)家族(CD63^a、CD81^b、^c和CD9^c、^d)。此家族中的蛋白質具有四個跨膜區域，並且定位於細胞膜中富含四跨膜蛋白的微區域(Tetraspanin Enriched Microdomains, TEMs)。

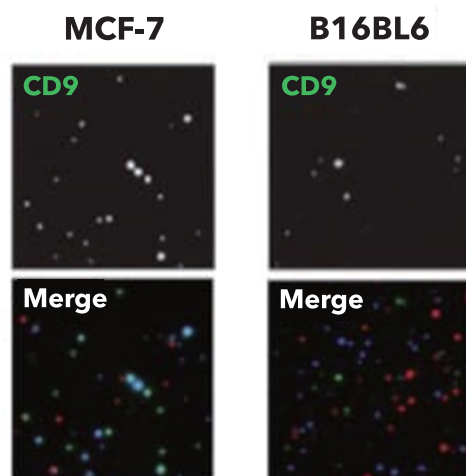
Tetraspanins
(CD9, CD63, CD81, CD82)



Markers	Function
CD9	與黏附分子(adhesion molecules)、跨膜受體和細胞內
CD81	訊息蛋白相關，以促進和調控訊息傳遞。
CD63	
CD82	

貨號	品名	內容物
NBP3-11740	Exosome Detection (Western Blot) Antibody Pack	NBP1-47546SS: Calnexin Antibody (3H4A7) NB100-65805SS: CD81 Antibody (1D6) - BSA Free MAB1663-SP: HSP70/HSPA1A Antibody (242707) [Unconjugated] MAB1455-SP: Albumin Antibody (188835) [Unconjugated] - Serum NBP2-42225SS: CD63 Antibody (H5C6) - BSA Free NBP2-77452SS: TSG101 Antibody - BSA Free MAB25292-SP: CD9 Antibody (1021007) [Unconjugated]

當全反射螢光(Total internal reflection fluorescence, TIRF)顯微鏡對單一囊泡進行成像時，研究人員發現四跨膜蛋白的定位取決於細胞來源。他們的結果及其他研究數據均顯示四跨膜蛋白的表現並不統一，並且每種細胞類型都分泌不同的EVs亞群。CD9和CD81傾向於在表現在同一個囊泡上，而CD63更常單獨存在。



Single vesicle imaging by TRIF of EVs isolated from the human breast cancer cell line MCF-7 and the mouse melanoma cell line B16BL6. Immobilized vesicles were stained with Mouse Anti-Human CD9 (MEM-61) (NB500-327) in green in merged image. Merged image shows co-localization of CD81 and CD9 (blue) on individual vesicles. Image was adapted from Han C, et al. Single-vesicle imaging and co-localization analysis for tetraspanin profiling of individual extracellular vesicles. J Extracell Vesicles. 2021 Jan;10(3):e12047. Access provided by Creative Commons License.

General Exosome Markers: Multivesicular Body (MVB)-Associated Proteins

在胞內體(Endosomal)成熟過程中，多囊體(Multivesicular Bodies, MVB)和腔內囊泡(Intraluminal Vesicles, ILVs)的形成需要非常特殊的細胞機制。MVB的形成受複雜的蛋白質複合物調控，這些複合體統稱為胞內分選轉運複合物(Endosomal Sorting Complex Required for Transport, ESCRT)。因此，ESCRT相關蛋白建議可以作為外泌體的標記物，在細胞內囊泡生成途徑的應用目前還有待證明。

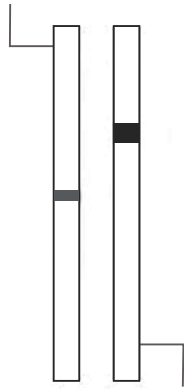
MVB making proteins

Alix, Tsg101, Clathrin Ubiquitin

Markers	Function
Alix TSG101	與參與細胞質分裂和膜斷裂的中體(Midbody)蛋白質相互作用
Flotillin-1	胞膜窖(Calveolae)內的支架蛋白，參與胞膜窖或類胞膜窖囊泡的形成
Clathrin	參與受器媒介的胞吞作用(Receptor Mediated Endocytosis)和早期胞內體的

Catalog	Product	Reactivity	Applications
NBP1-90201	Alix Antibody	Hu, Mu, Rt	WB, ICC/IF, IHC, IHC-P, KD
NB200-112	TSG101 Antibody (4A10)	Hu, Mu, Rt, Po, Ca, Ha, Pm, Pm	WB, ELISA, EM, Flow, ICC/IF, IHC, IHC-P, IP, IHC-FrFI, KD
NBP1-79022	Flotillin-1 Antibody	Hu, Mu, Po, Bv, Pm, Pm-Or	WB, Simple Western, IP
NB300-613	Clathrin Heavy Chain 1/CHC17 Antibody (X22) - BSA Free	Hu, Mu, Rt, Bv, Ha, Pm	WB, Flow, ICC/IF, IHC, IHC-P, IM, IP

Control : TSG101



Control : HSP90

Simple western lane view (acquired with Wes) showing Rabbit Anti-Human TSG101 (NBP-267884), and Rabbit Anti-Human HSP90 (NBP2-67395) on EVs isolated by ultracentrifugation. Image adapted from Haney MJ, Zhao Y, Jin YS, Batrakova EV. Extracellular Vesicles as Drug Carriers for Enzyme Replacement Therapy to Treat CLN2 Batten Disease: Optimization of Drug Administration Routes. *Cells*. 2020;9(5):1273. doi:10.3390/cells9051273. Access provided by Creative Commons License.

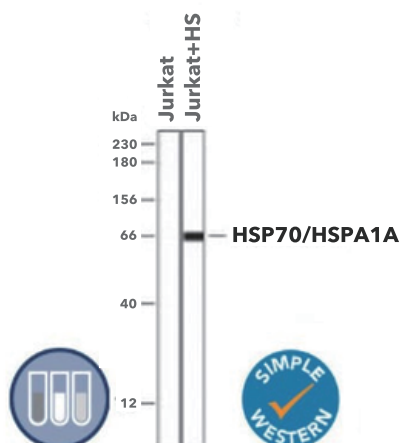
Role of Chaperone Proteins in Exosomes

熱休克蛋白(Heat-shock proteins, HSP)是屬於細胞內分子伴隨蛋白家族，因應細胞內壓力而產生。研究最多的是HSP60、HSP70和HSP90。由於細胞感受到壓力的時候外泌體的分泌通常會增加，例如癌症、發炎反應和缺氧，因此經常發現HSP被併入外泌體中。HSP70在大多數癌症中都有表達，研究表明HSP70相關的EVs在腫瘤微環境中具有免疫抑制作用。

Markers	Function
HSP90 HSP60 HSC70 HSP70	確保蛋白質正確折疊並防止異常蛋白質聚合體(Aggregates)在細胞內積聚。

Catalog	Product	Reactivity	Applications
NB100-1972	HSP90 Antibody (AC88)	Hu, Mu, Rt, Po, Ba, Ca, Ch, Fi, Gp, Pm, Rb, Sh	WB, Flow, Flow-IC, ICC/IF, IHC, IHC-P
NBP1-77397	HSP60 Antibody - BSA Free	Hu, Mu, Rt, Bv, Ch, Ha, Pm-Or	WB, Simple Western, Flow, IB, ICC/IF, IHC, IHC-P, KD
NB120-2788	HSPA8/HSC71/Hsc70 Antibody (13D3)	Hu, Mu, Rt, Bv, Fe, Fi, Ha, Pm	WB, Flow, IB, ICC/IF, IHC, IHC-Fr, IHC-P, IP, B/N
NB110-61582	HSP70/HSPA1A Antibody (C92F3A-5)	Hu, Mu, Rt, Po, Bv, Ca, Ce, Ch, Dr, Fi, Gp, Ha, Pm, Rb, Sh	WB, ELISA, EM, Flow, ICC/IF, IHC, IHC-P, IM, MiAr, Binding Activity

HSP70

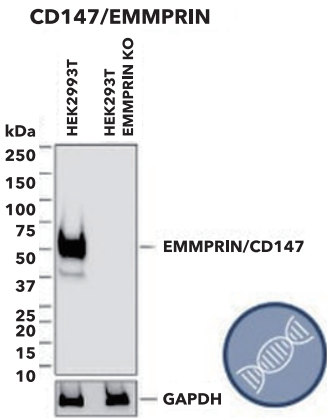


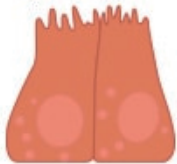
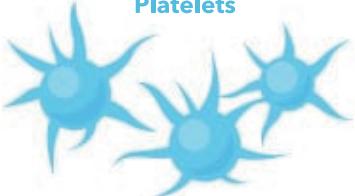
Simple Western lane view showing lysates of Jurkat human acute T cell leukemia cell line untreated (-) or treated (+) by heat shock (HS) and stained with Mouse Anti-Human HSP70/HSPA1A (MAB1663). Band appears around 70 kDa in HS but not untreated cells. The biological strategies badge shows antibody specificity using biological strategies (heat-shock) and detection of protein only in treated cells."

Tissue/Cell Type-Specific Exosome Markers

蛋白質標記對於辨別EVs的細胞來源也很重要。正如個別細胞類型表現特定標記物一樣，源自這些細胞的外泌體通常表現該細胞類型的特徵蛋白。例如，CD147/EMMPRIN在卵巢癌腫瘤細胞產生的EV上表現。

Western blot lysates of HEK293T human embryonic kidney parental cell line and EMMPRIN/CD147 knockout (KO) HEK293T cell line. PVDF membrane was probed with Goat Anti-Human EMMPRIN/CD147 (AF972), followed by HRP-conjugated Rabbit Anti-Goat IgG Secondary Antibody (HAF017). GAPDH (AF5718) is shown as a loading control. The Genetic strategies badge shows antibody specificity by comparing parental cell line to KO cell line.



Cell Type	Exosome Marker
Epithelial Cells 	EpCAM ^e CD147/EMMPRIN EGFR
Lymphocytes (B cells, T cells, NK cells) 	CD3 CD19 CD20 CD37 NKG2D CD56
Mesenchymal Stem/Stromal Cell (MSCs) 	MMP-9 CD44 CD73 HGF
Platelets 	CD31/PECAM-1 CD41 CD62p PF4 GP-IX(CD42a)
Neurons 	L1CAM GluR2 NF-L TDP43

Catalog	Product	Reactivity	Applications
NB600-1182	EpCAM/TROP1 Antibody (AUA1) - BSA Free	Hu	WB, ELISA, Flow, ICC/IF, IHC, IHC-P, CyTOF-ready
NB500-430	EMMPRIN/CD147 Antibody (MEM-M6/1) - BSA Free	Hu, Eq	WB, ELISA, Flow, IHC, IHC-P, IP, CyTOF-ready
NBP1-84814	EGFR Antibody	Hu, Mu, Rt	WB, ICC/IF, IHC, IHC-P
NB600-1441	CD3 Antibody (SP7)	Hu, Mu, Po, Ca	Flow, ICC/IF, IHC, IHC-Fr, IHC-P, KD
NBP2-25196	CD19 Antibody (CB19) - BSA Free	Hu, Mu	WB, Flow, ICC/IF, In vitro, CyTOF-ready
NBP2-45454	CD20 Antibody (OTI4B4)	Hu, Mu, Ca, Pm	WB, IHC, IHC-P
NBP1-28869	CD37 Antibody (2B8)	Hu, Mu	ELISA, Flow, ICC/IF, IHC, IHC-P
NB100-65956	NKG2D/CD314 Antibody (1D11) - Low Endotoxin, Azide and BSA Free	Hu	Flow, ICC/IF, IHC, IHC-Fr, IHC-P, IP, CyTOF-ready, Func
NBP2-15184	NCAM-1/CD56 Antibody (123C3.D5)	Hu, Rt	Simple Western, Flow, Flow-CS, ICC/IF, IHC, IHC-P
NBP2-13173	MMP-9 Antibody (4A3)	Hu, Mu, Rt, Po, Ca, Eq, Pm, Rb	WB, ELISA, IHC, IHC-P
NBP1-47386	CD44 Antibody (8E2F3) - BSA Free	Hu, Mu, Rb(-), Pm, Ca, Eq	WB, ELISA, Flow, Flow-IC, ICC/IF, IHC, IHC-P, IP
NBP2-48480	5'-Nucleotidase/CD73 Antibody (AD2) - BSA Free	Hu	Flow, Flow-CS, IHC, IHC-Fr, IHC-P
NBP1-19182	HGF Antibody (7-2) - BSA Free	Hu, Mu	WB, ICC/IF, IHC, IHC-P
NB100-2284	CD31/PECAM-1 Antibody	Hu, Mu, Rt, Po, Ca	WB, ICC/IF, IHC, IHC-Fr, IHC-P
NBP1-84581	Integrin alpha 2b/CD41 Antibody	Hu	WB, Simple Western, IHC, IHC-P
NB100-65392	P-Selectin/CD62P Antibody (Psel.KO.2.7) - BSA Free	Hu, Mu, Rt, Bv, Eq, Fe, Gt, Sh	Flow, ICC/IF, IHC, IHC-Fr, IHC-P, IP
NBP3-16251	CXCL4/PF4 Antibody (6Q10H2)	Hu, Mu, Rt	WB, ICC/IF, IHC, IHC-P
NBP3-03507	GP-IX Antibody	Hu, Mu, Rt	WB, IHC, IHC-P
NB100-2682	L1CAM Antibody (UJ127.11) - BSA Free	Hu, Mu	WB, ELISA, Flow, Flow-CS, ICC/IF, IHC, IHC-Fr, IHC-P, IP, CyTOF-ready, ICC/IF
NBP2-75510	GluR2 Antibody (JU20-43)	Hu, Mu, Rt	WB, ICC/IF, IHC, IHC-P, IP
NB300-131	NF-L Antibody	Hu, Mu, Rt, Po, Bv	WB, ICC/IF, IHC, IHC-P, KO
NB110-55376	TDP-43/TARDBP Antibody - BSA Free	Hu, Mu, Ch, Pm, Xp, Ze	WB, ELISA, ICC/IF, IHC, IHC-P

貨號	品名	Sample Type	Applications:
NBP3-14803	Immunoplate for neural derived exosome isolation (Colorimetric)	Human Plasma	ELISA, IA
NBP3-14802	Immunoplate for glial derived exosome isolation (Colorimetric)		
NBP3-14806	Immunoplate for Tumor-derived Exosome Isolation (Colorimetric)		
NBP3-14805	Immunoplate for total Exosome Isolation (CD63, Colorimetric)		
NBP3-14804	Immunoplate for total Exosome Isolation (CD9, Colorimetric)		

Markers of Exosome Function

EVs執行廣泛的功能，EVs攜帶的內容物也同樣具有多樣性。具體內容物取決於宿主物種、細胞來源、生物體液和疾病狀態。下面列出了一些常見的訊息傳遞、代謝、細胞死亡和免疫調節標記物：

Function	Markers
Signaling	HIF-1 α ^f β -catenin VEGF CDC42 ARF1
Metabolic Enzymes	GAPDH PGK1
Cell death/cytotoxicity	FasL Granzyme A Granzyme B
Immunomodulatory	HLA-A/B/C HLA-DR/DP/DQ TNF- α CD86 Galectin-9 TGF- β PD-L1 ^e

詳細資訊請參考
Novus網頁



Catalog	Product	Reactivity	Applications
NB100-105	HIF-1 alpha Antibody (H1alpha67)	Hu, Mu, Rt, Po, Bv, Ca, Fe, Ma, Pm, Pm, Rb, Sh, Xp	WB, Simple Western, ChIP, ELISA, Flow, GS, IA, IB, ICC/IF, IHC, IHC-Fr, IHC-P, In vitro, IP, LA, PLA, TCS, IHC-FrFI, KD, KO
NBP1-54467	beta-Catenin Antibody (12F7) - BSA Free	Hu, Mu, Rt, Av-Du, Ch, Pm	WB, Simple Western, Flow, Flow-IC, ICC/IF, IHC, IHC-P, IP
NB100-664	VEGF Antibody (VG1) - BSA Free	Hu, Mu, Rt, Po, Ca	WB, Simple Western, ELISA, Flow, ICC/IF, IHC, IHC-Fr, IHC-P, CyTOF-ready
NBP2-67895	CDC42 Antibody (JJ086-04)	Hu, Mu, Rt	WB, Flow, IHC, IHC-P, IP
NB300-505	ARF1 Antibody (1D9)	Hu, Mu, Rt, Bv, Ca	WB, ELISA, Flow, ICC/IF, IHC, IHC-P, IP
NB300-221	GAPDH Antibody (1D4)	Hu, Mu, Rt, Po, Av, Bv, Ca, Ch, ChHa, Dr, Eq, Fe, Ha, Ma-Op, Pm, Rb, Sh, Ze	WB, Simple Western, ChIP, ICC/IF, IHC, IHC-Fr, IP, KD, Single-Cell Western
NBP2-19784	PGK1 Antibody	Hu, Mu, Rt, Ye, Po, Bv, Pm, RM, Sh	WB, ELISA, ICC/IF, IHC, IHC-P, IP, PLA
NBP1-97519	Fas Ligand/TNFSF6 Antibody (A11)	Mu	Flow, IHC, IHC-Fr, IHC-P
NBP2-16736	Granzyme A Antibody	Hu	WB, IHC, IHC-P
NBP2-59678	Granzyme B Antibody - BSA Free	Hu	WB, Flow, IHC, IHC-P
NB100-64775	HLA ABC Antibody (W6/32) - Azide and BSA Free	Hu, Mu, Bv, Pm, Pm	ELISA, Flow, ICC/IF, IHC, IHC-Fr, IP, CyTOF-ready, IHC-P (-)
NBP2-79709	HLA DQ/DR/DP Antibody (HLA-Pan/2967R)	Hu	WB, ELISA, Flow, ICC/IF, IHC, IHC-P, PA
NBP1-19532	TNF-alpha Antibody - BSA Free	Hu, Mu, Rt, Ca, Fi, Pm, Fe, Pm, Pm	WB, Flow, ICC/IF, IHC, IHC-Fr, IHC-P, IHC-FrFI
NBP2-25208	B7-2/CD86 Antibody (BU63)	Hu, Mu, Rt	WB, Flow, ICC/IF, IHC, IHC-Fr, IHC-P, KO
NBP2-45619	Galectin-9 Antibody (OTI1D12)	Hu, Mu	WB, IHC, IHC-P
NBP2-45137	TGF-beta Antibody (1D11.16.8) - BSA Free	Hu, Mu, Rt, Bv, Ca, Ha, Pm	ICC/IF, IHC, IHC-P, S-ELISA, ELISA(Cap)
NBP1-76769	PD-L1 Antibody - BSA Free	Hu, Mu, Rt	WB, ELISA, Flow, ICC/IF, IHC, IHC-Fr, IHC-P, IP, Dual ISH-IHC, ICC/IF, IHC-WhMt, KD

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