

# Exosome 方程式



SeraMir  
+  
miRNA qPCR

EXOCET/  
EXOELISA

Exo-Flow IP

Antibody

定量 exosome

抓住特定族群 exosome

研究 exosome 蛋白質表現

研究 exosome miRNA 表現



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# Exosome 方程式

ExoQuick + EXOCET/ELISA

定量 exosome

P.03

ExoQuick + Exo-Flow IP

抓住特定族群 exosome

P.08

ExoQuick + Antibody

研究 exosome 蛋白質表現

P.10

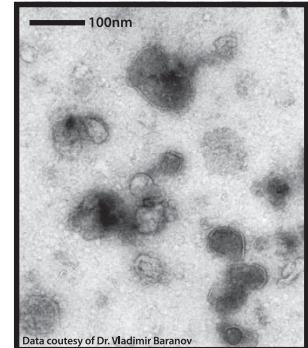
ExoQuick + SeraMir + miRNA qPCR

研究 exosome miRNA 表現

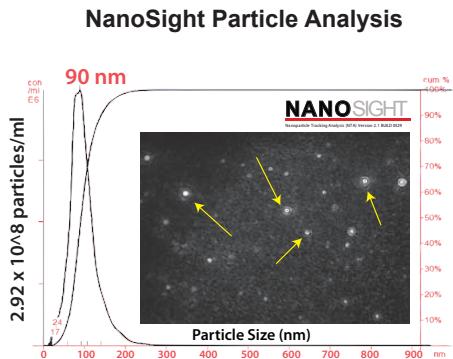
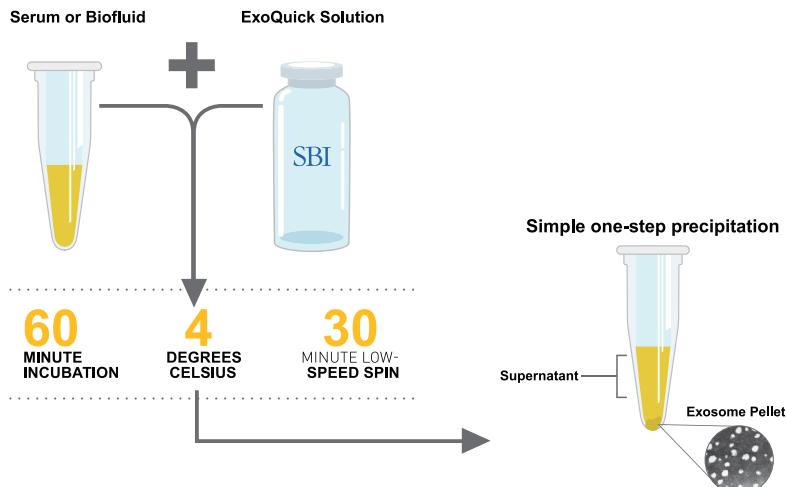
P.12

# 分離濃縮 exosome ExoQuick™

- 不需費時超高速離心
- 不需額外買 DynaBeads 及抗體
- 適用多種樣本：Serum、Ascites fluid、Urine、Spinal fluid、Culture media、Saliva, etc
- 操作簡單，一小時內搞定
- 回收 exosomes 量最多
- 分離完整且具有生物活性 exosomes，後續可進行 functional studies



以電子顯微鏡觀察 exosomes。



以 NanoSight LM10 分析 ExoQuick 濃縮的 exosomes，確認其完整性及濃度。

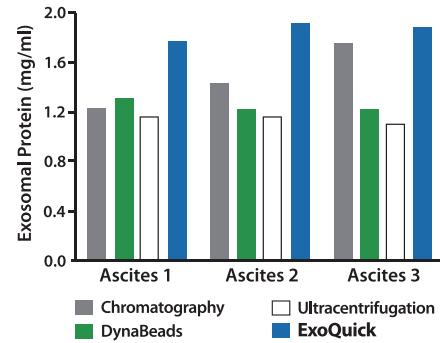


# 分離濃縮 exosome ExoQuick™

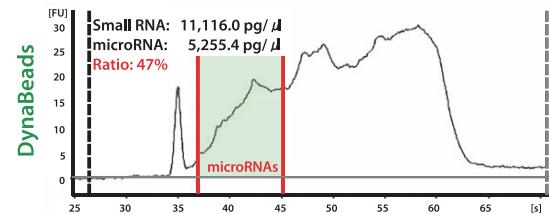
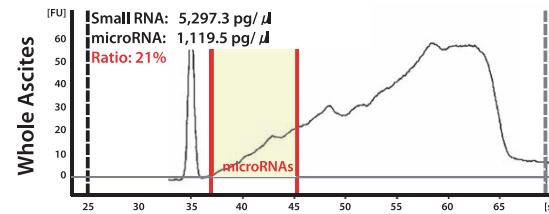
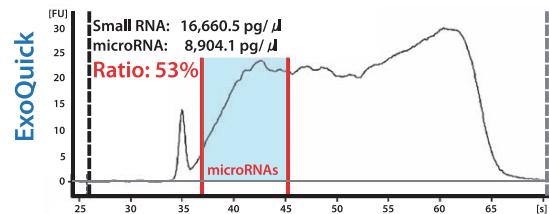
## 比較 exosomal protein、microRNA 產量

利用 ExoQuick、chromatography、ultracentrifugation 及 anti-EpCAM magnetic DynaBeads 純化 exosomes

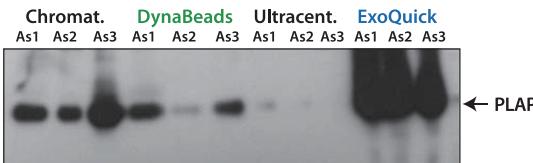
ExoQuick-萃取最多量的 exosomal proteins



ExoQuick-萃取最多量的 exosomal microRNAs



Placental Alkaline Phosphatase Biomarker Enrichment



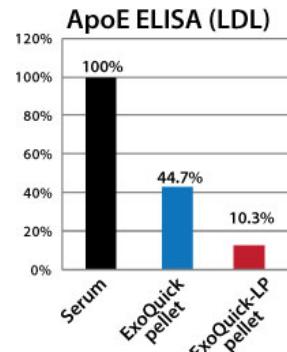
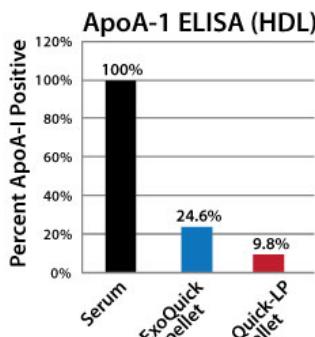
Placental Alkaline Phosphatase Biomarker Enrichment

# 分離濃縮 exosome ExoQuick-LP

## ExoQuick-LP

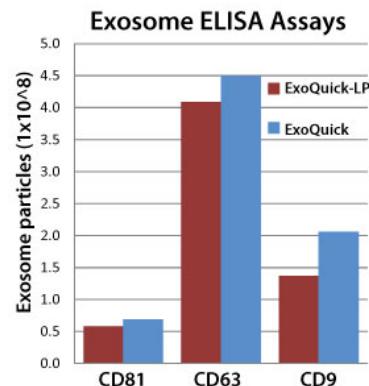
- 去除血清或血漿中的 lipoprotein，避免這些 particles 中 miRNA 干擾研究 exosome 相關的 miRNA
- 使用 pre-clearing reagents，有效移除血清或血漿中的 lipoprotein particles，再使用 ExoQuick，就能輕鬆分離其中的 exosomes

可大量去除 HDL 和 LDL 中的 lipoprotein particles



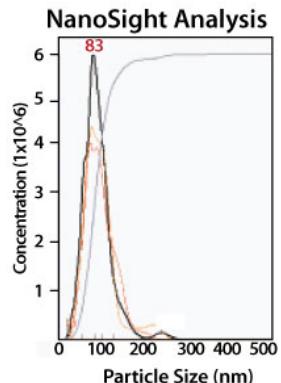
以 ELISA 證明 ExoQuick-LP 能大量去除 HDL 和 LDL 中的 lipoprotein particles。

不會造成 exosomes 的損失



以 ELISA 測量 exosomes 數目，證明 ExoQuick-LP 不會造成 exosomes 的損失。

不影響 exosomes 完整度



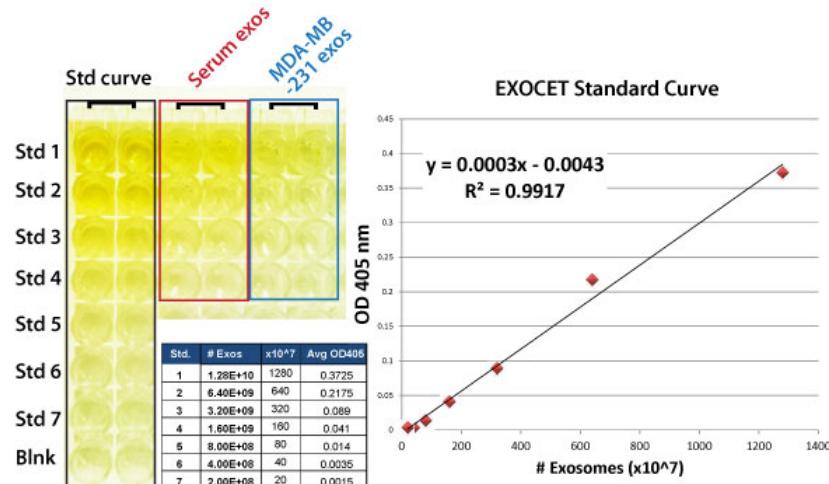
以 NanoSight tracking analysis 測量使用 ExoQuick-LP 分離的 exosomes 大小，證明 ExoQuick 不會影響 exosomes 完整度。

# 定量 exosome

## ExoQuick™ + EXOCET/ExoELISA

### EXOCET

- 直接測量 Acetyl-CoA Acetylcholinesterase (AChE) activity
- AChE 已被證實存在於 serum、stem cell、cancer cell line 及 MSC exosomes
- Human、mouse、rat exosomes 都適用
- 以 OD405 讀取數值，輕鬆定量
- 只需 20 分鐘即可完成
- 附有 standard ( 經 NanoSight 分析過 )，準確定量

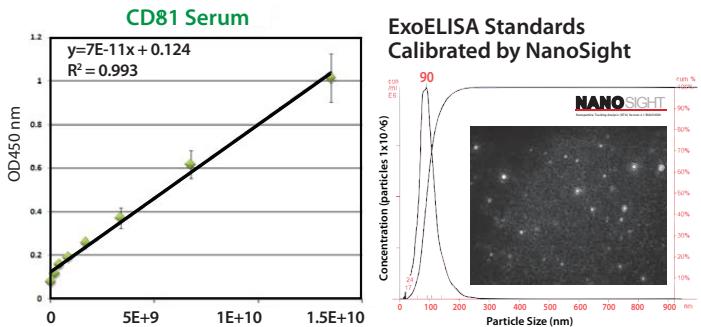
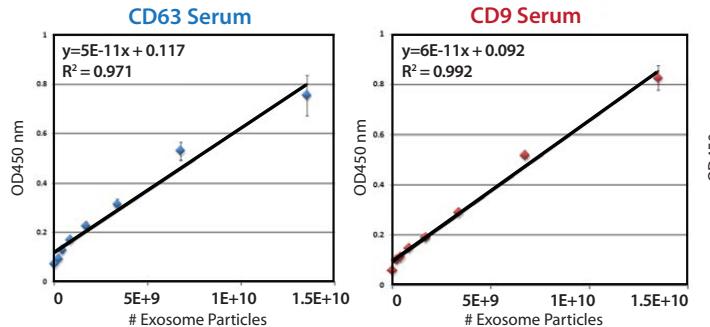
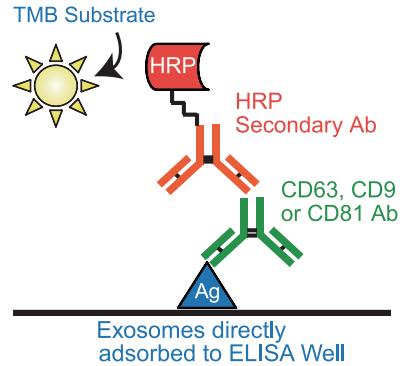


# 定量 exosome

## ExoQuick™ + EXOCET/ExoELISA

### ExoELISA

- 提供三種 ELISA kits : CD63、CD9、CD81
- 每組均附 protein standard，可進行蛋白定量
- 為 96-well ELISA plate 型式 ( 12 條 8 連排 )，操作方便



# 抓住特定族群的 exosome **ExoQuick™ + Exo-Flow IP**

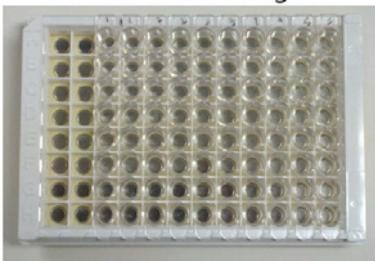
## Exo-Flow96 and 32 Exosome IP

- 利用 CD9、CD63、CD81 進行 exosome immunopurification
  - 快速且高通量純化 exosomes (一次純化 32 或 96 個樣本)
  - 磁珠表面積大，抓取 exosomes 的效率極高
  - 能直接從血清或血漿純化 exosomes，不需事先濃縮或分離 exosomes
  - 純化後的 exosomes 能再被完整 elute 出來，方便進行 functional studies 及 exoRNA 和 protein biomarker analysis

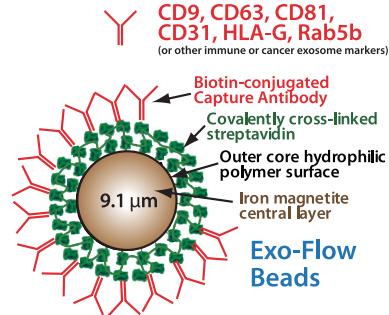
### Plate Magnet alone



## Clear Plate on Magnet



## Structure and Gating of Exo-Flow Beads

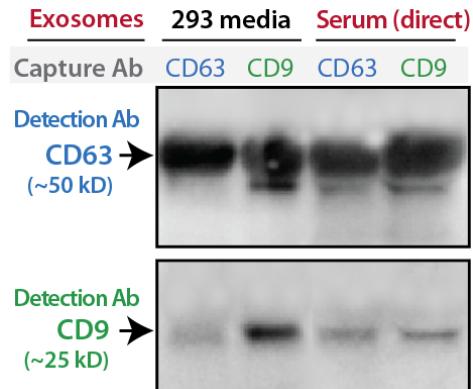


可明顯觀察到結合於磁珠的 **exosomes**，操作方便。

## Exosome Bead Capture Photo

The figure shows a 2x6 grid of petri dishes. The top row is labeled "293 media" and the bottom row "Serum (direct)". The columns are labeled "CD9", "CD63", and "CD81". In each dish, a small white pellet at the bottom indicates isolated exosomes. Arrows point from the labels "Exosomes on magnetic beads" to the bottom of the first and second columns of each row.

# 抓住特定族群的 exosome ExoQuick™ + Exo-Flow IP



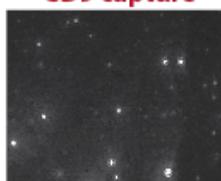
## Western Blot Analysis

以 Western blot 分析使用 Exo-Flow IP kit 純化的 HEK 293 media exosomes 及 serum exosomes。

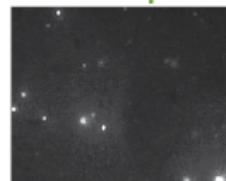
293 Exosomes  
CD81 capture



Serum Exosomes  
CD9 capture



Serum Exosomes  
CD63 capture



## NanoSight Exosome Analysis

使 用 Exo-Flow IP kit 純 化 HEK 293 media exosomes (concentrated by ExoQuick-TC) 及 serum exosomes，以 NanoSight analysis 分析 exosomes 大小及數目。

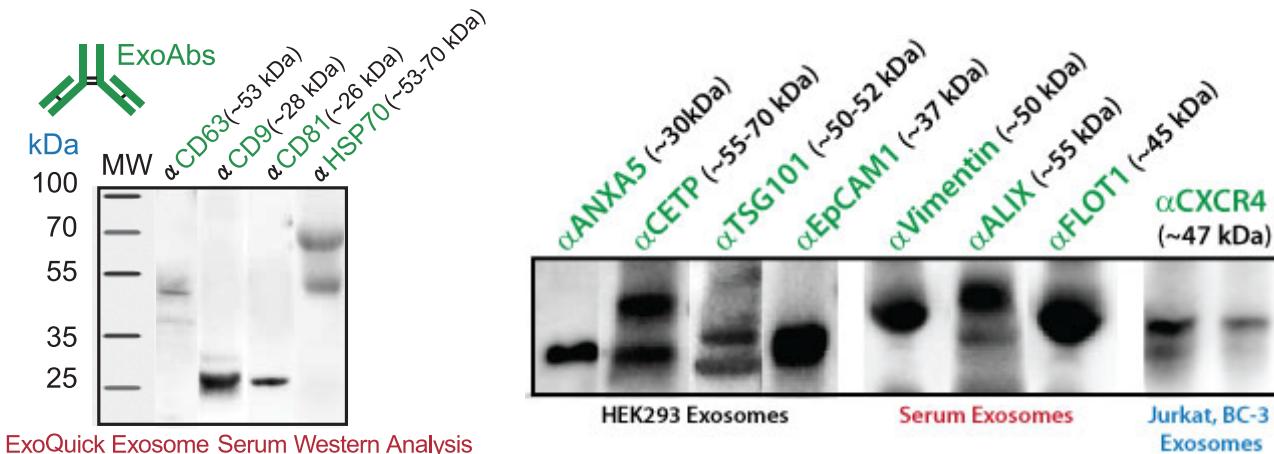


# 研究 exosome 蛋白質表現 ExoQuick™ + Antibody

## Exosome Antibody

- 提供 Western blots 專用的 anti-exosome marker antibody
- Exosome marker antibody : CD63、CD9、CD81、Hsp70、CETP、ANXA5-1、TSG101、EpCam、Vimentin、ALIX、FLOTILLIN-1 及 CXCR4
- 每組抗體均附二抗 (goat anti-rabbit IgG HRP)

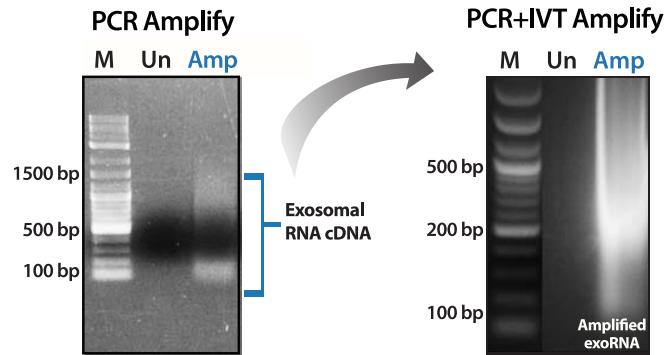
exosome markers and tissue specific exosome markers



# 研究 exosome miRNA 表現 ExoQuick™ + SeraMir + miRNA qPCR

## SeraMir™ Exosome RNA Amplification Kit

- 以 phenol-free lysis buffer 及 spin column 純化 exoRNA
- 降低變異性：以 ExoQuick 分離 exosomes
- 增加敏感度：放大 exoRNA，以進行 qPCR
- 獲得更多 data：以 T7 IVT amplified sense exoRNA 進行 microarrays 或 NextGen Sequencing

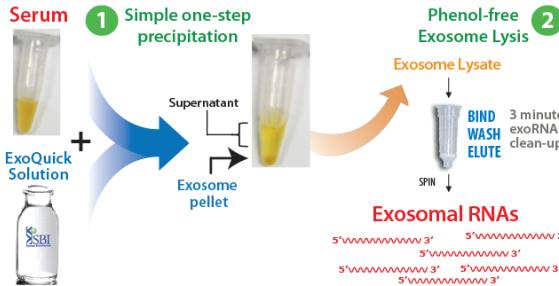


左圖：利用 PCR 反應放大 exoRNA cDNA 產量。

右圖：利用 T7 in vitro transcription 放大 sense exoRNAs，後續可做 Microarrays 以及 NextGen Sequencing。

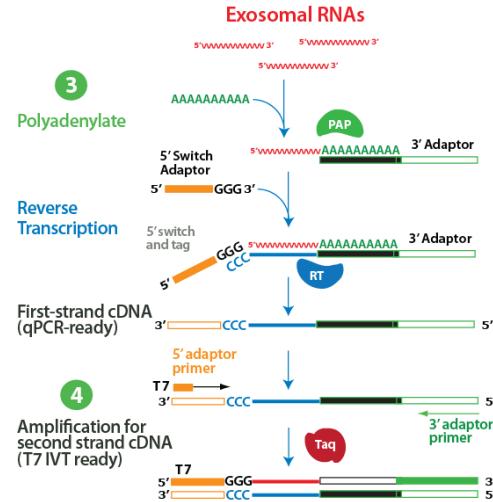
# 研究 exosome miRNA 表現 ExoQuick™ + SeraMir + miRNA qPCR

利用 SeraMir Exosome RNA Purification Kit  
純化 serum exoRNAs



- 1 利用 ExoQuick one-step precipitation，濃縮 serum exosomes。
- 2 利用 phenol-free lysis buffer 及 spin columns，純化 exosome RNAs (exoRNAs)。
- 3 利用 polyA polymerase 於 exoRNA 3' end 添加 polyA tail；再添加 3' Adaptor、5' Switch Adaptor 及 RTase，進行反轉錄並形成 cDNA。此時的產物可進行 qPCR 相關實驗。
- 4 若想放大 cDNA 產量，可利用 SeraMir PCR amplification primer mix 以及 Taq polymerase，進行 PCR 反應。  
若想放大 sense exoRNAs 產量，可利用放大後 cDNA 以及 T7 RNA polymerase，進行 *in vitro* transcription。

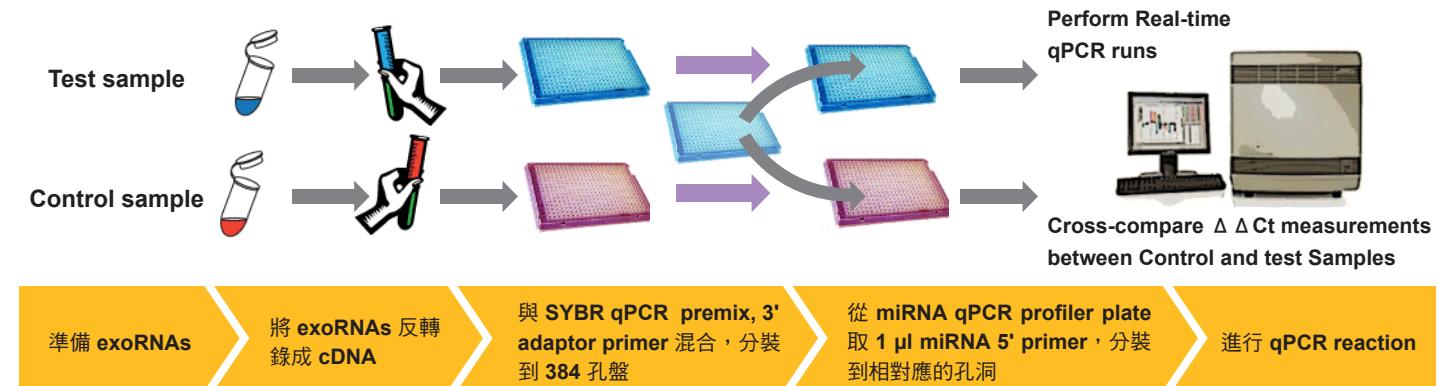
利用 SeraMir Exosome RNA Amplification Kit，  
將 exoRNAs 反轉錄為 cDNA



# 研究 exosome miRNA 表現 ExoQuick™ + SeraMir + miRNA qPCR

## Exosome miRNA qPCR Profiling kit

- 利用 qPCR 反應，精準定量 miRNA 含量
- 內含 380 種 miRNAs，三種 control
- 內含 excel 版軟體，數據分析方便



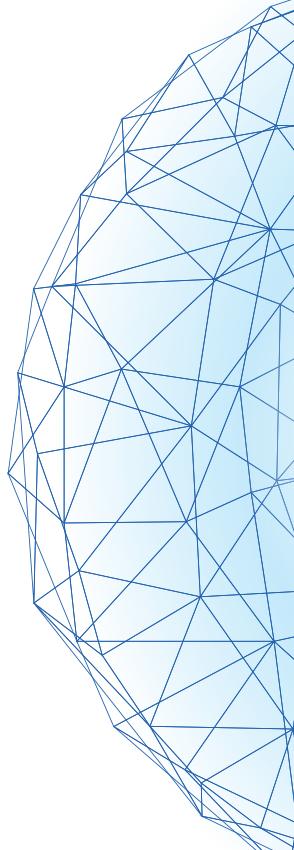
	Cat No	Description
<b>Isolation</b>		
ExoQuick	EXOQ5A-1	ExoQuick exosome precipitation solution (5 ml)
	EXOQ20A-1	ExoQuick exosome precipitation solution (20 ml)
	EXOQ5TM-1	ExoQuick Plasma prep and Exosome precipitation kit (5 ml ExoQuick plus 500 ul Thrombin at 500U/mL)
ExoQuick-TC	EXOTC10A-1	ExoQuick TC 10ml
	EXOTC50A-1	ExoQuick TC 50ml
ExoQuick-LP	EXOLP5A-1	ExoQuick-LP for lipoprotein pre-clear & exosome isolation
<b>Purification</b>		
Exo-Flow IP	EXOFLOW32A-CD63	Exo-Flow32 CD63 IP kit
	EXOFLOW32A-CD81	Exo-Flow32 CD81 IP kit
	EXOFLOW32A-CD9	Exo-Flow32 CD9 IP kit
	EXOFLOW32A-Tetra	Exo-Flow32 Tetra IP kit (CD9, CD63, CD81)
	EXOFLOW96A-CD63	Exo-Flow96 CD63 IP kit
	EXOFLOW96A-CD81	Exo-Flow96 CD81 IP kit
	EXOFLOW96A-CD9	Exo-Flow96 CD9 IP kit
	EXOFLOW96A-Tetra	Exo-Flow96 Tetra IP kit (CD9, CD63, CD81)
<b>Quantitation</b>		
EXOCET	EXOCET96A-1	EXOCET Exosome Quantitation Kit
ELISA	EXOEL-CD63A-1	Exosome ELISA Complete Kit (CD63 detection) 96 rxns
	EXOEL-CD81A-1	Exosome ELISA Complete Kit (CD81 detection)
	EXOEL-CD9A-1	Exosome ELISA Complete Kit (CD9 detection) 96 reactions

Cat No	Description
Detection	
Antibody	EXOAB-CD63A-1 Anti-CD63 Antibody (rabbit anti-human) with goat anti-rabbit HRP secondary antibody
	EXOAB-CD81A-1 Anti-CD81 Antibody (rabbit anti-human) with goat anti-rabbit HRP secondary antibody
	EXOAB-CD9A-1 Anti-CD9 Antibody (rabbit anti-human) with goat anti-rabbit HRP secondary antibody
	EXOAB-Hsp70A-1 Anti-Hsp70 Antibody (rabbit anti-human) with goat anti-rabbit HRP secondary antibody
	EXOAB-KIT-1 ExoAb Antibody Kit (CD9, CD63, CD81, Hsp70 antibodies, rabbit anti-human) with goat anti-rabbit HRP secondary antibody
	EXOAB-CETP-1 Anti-CETP Antibody (polyclonal rabbit anti-human, mouse, rat) with goat anti-rabbit HRP secondary antibody
	EXOAB-FLOT1-1 Anti-FLOT1 Antibody (rabbit anti-human, mouse, rat) with goat anti-rabbit HRP secondary antibody
	EXOAB-EPCAM1-1 Anti-EpCam Antibody (rabbit anti-human, mouse, rat) with goat anti-rabbit HRP secondary antibody
	EXOAB-TSG101-1 Anti-TSG101 Antibody (rabbit anti-human, mouse, rat) with goat anti-rabbit HRP secondary antibody
	EXOAB-CXCR4-1 Anti-CXCR4 Antibody (rabbit anti-human, mouse, rat) with goat anti-rabbit HRP secondary antibody
	EXOAB-VMTN-1 Anti-Vimentin Antibody (rabbit anti-human, mouse, rat) with goat anti-rabbit HRP secondary antibody
	EXOAB-ALIX-1 Anti-ALIX Antibody (rabbit anti-human, mouse, rat) with goat anti-rabbit HRP secondary antibody
	EXOAB-ANXA5-1 Anti-ANXA5 Antibody (rabbit anti-human, mouse, rat) with goat anti-rabbit HRP secondary antibody
	EXOAB-POS-1 Human Exosome lysate positive protein control (10ug/ul), Size 50 ul
Discovery	
SeraMir	RA800A-1 SeraMir Exosome RNA Ampfication Kit
	RA800TC-1 SeraMir Exosome RNA Amplification kit from Media and Urine (10ml ExoQuick-TC and 10 exoRNA columns)
	RA806A-1 SeraMir Exosome RNA Purification kit (5ml ExoQuick and 20 exoRNA columns)
	RA806TC-1 SeraMir Exosome RNA Purification kit (10ml ExoQuick-TC and 10 exoRNA columns) 10 preps
miRNA qPCR	RA810A-1 SeraMir Exosome RNA 384 microRNA qPCR Profiler Assay Set
	RA811A-1 Mouse SeraMir Exosome RNA 384 microRNA qPCR Profiler Assay Set (20 assays)





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