invitrogen

Take a closer look at exosomes

Vesicle transport and cell signaling
The 2013 Nobel Prize™ in Physiology or Medicine was jointly awarded to three scientists for their discovery of vesicle transport in cells, a clear indication of the importance of vesicles in physiology and their applied potential in diagnostics and therapy.

Exosomes and other extracellular vesicles are powerful mediators and strong biomarker candidates. There's an urgent need for the development of new methods for vesicle isolation and characterization.

To answer this need, we're continually expanding our product offerings. The products presented here can help simplify the overall study of exosomes, and are also applicable to translational research.

An up-to-date list of published scientific papers citing the use of these products can be found at **thermofisher.com/exosomes**

"Exosomes: the next small thing"
We asked ten prominent scientists to share their thoughts on the field of exosome research. Based on these interviews, a fascinating six-part video documentary was produced. This miniseries tells the story of exosome research and its impact on important research areas such as cancer, immunology, stem cell research, and potential future therapeutic and diagnostic applications.

Watch the six-part video series at
thermofisher.com/exosomesdocumentary

ExosomesTalk
Join the conversation and post questions at **exosomestalk.com**. This forum is curated by scientists with substantial experience working with exosomes.

ThermoFisher
SCIENTIFIC

Exosome isolation

The total exosome isolation reagents allow for efficient precipitation and high recovery of intact exosomes (Figure 1).

- Typically 15–20 minutes of hands-on time
- A lot easier and less tedious than ultracentrifugation

Ordering information

Starting sample for total exosome isolation	Cat. No.
Cell culture media (100 mL)	4478359
Blood serum (30 mL)	4478360
Blood plasma (20 mL)	4484450
Urine (50 mL)	4484452
Other body fluids (up to 30 mL)	4484453

By targeting classical exosome surface proteins, Dynabeads™ magnetic separation technology allows you to easily pull out specific exosome subpopulations (preenriched from cell culture) (Figure 2).

- Scalable protocol with minimal hands-on time
- Enables you to obtain a highly pure exosome subset

Ordering information

Subpopulation isolation	Quantity	Cat. No.
Human CD9 isolation	50 preps	10614D
Human CD81 isolation	50 preps	10616D
Human EpCam isolation	50 preps	10618D
Human CD63 (isolation/detection)	30 preps	10606D
Flexible streptavidin-based system (use your own biotinylated antibody)	30 preps	10608D

Exosome characterization

Preenriched exosomes can easily be visualized by flow cytometry while bound to the surface of Dynabeads™ magnetic beads, enabling the detection of specific exosomal markers.

- Clear and defined FSC/SSC for easier gating
- Typically less than 1 hour of hands-on time

Ordering information

Subpopulation analysis	Quantity	Cat. No.
Human CD9 detection	100 rxns	10620D
Human CD81 detection	100 rxns	10622D
Human EpCam detection	100 rxns	10624D
Human CD63 (isolation/detection)	150 rxns	10606D
Flexible streptavidin-based system (use your own biotinylated antibody)	150 rxns	10608D

Light microscopy can be used to visualize exosomes. Specialized spin columns can be used to remove unincorporated dyes.

- Remove low molecular-weight contaminants
- Allows for buffer exchange and desalting

Ordering information

Supporting products	Quantity	Cat. No.
BODIPY™ TR Ceramide	250 µg	D7540
SYTO™ RNASelect™ Green Fluorescent Cell Stain	100 µL	S32703
Exosome Spin Columns (for dye removal, MW 3000)	30 columns	4484449

Isolation of exosomal cargo

The Invitrogen™ Total Exosome RNA & Protein Isolation Kit allows for the isolation of total RNA—or both RNA and proteins—from your sample of preenriched exosomes.

- Highly pure total RNA (including small-RNA fraction)
- Recover protein and RNA from the same sample

Ordering information

Isolation of total RNA & proteins	Quantity	Cat. No.
Total Exosome RNA & Protein Isolation Kit	40 preps	4478545

By using fast and gentle Dynabeads magnetic separation technology, you can isolate proteins and protein complexes from preenriched exosomes—typically in only 30 minutes.

- 10–50X concentration of exosomal proteins
- Helps to significantly minimize background

Ordering information

Immunoprecipitation products	Quantity	Cat. No.
Exosome Immunoprecipitation Reagent (Protein A)	1 mL	10610D
Exosome Immunoprecipitation Reagent (Protein G)	1 mL	10612D

Analysis of exosomal cargo

Specific monoclonal antibodies allow for the detection of cellular and exosomal antigens (Figure 2). Exosomal RNA and membrane components can also be labeled using fluorescent dyes, while unincorporated dye can be removed by using specific spin columns (Figure 3).

- Antibodies verified for western analysis
- Remove low molecular weight (<3,000 MW) contaminants

Ordering information

Antibodies for western analysis	Quantity	Cat. No.
Anti–Human CD9	0.2 mL	10626D
Anti–Human CD63	0.2 mL	10628D
Anti–Human CD81	0.2 mL	10630D

Supporting products	Quantity	Cat. No.
BODIPY TR Ceramide	250 µg	D7540
SYTO RNASelect Green Fluorescent Cell Stain	100 µL	S32703
Exosome Spin Columns (for dye removal, MW 3,000)	30 columns	4484449

invitrogen

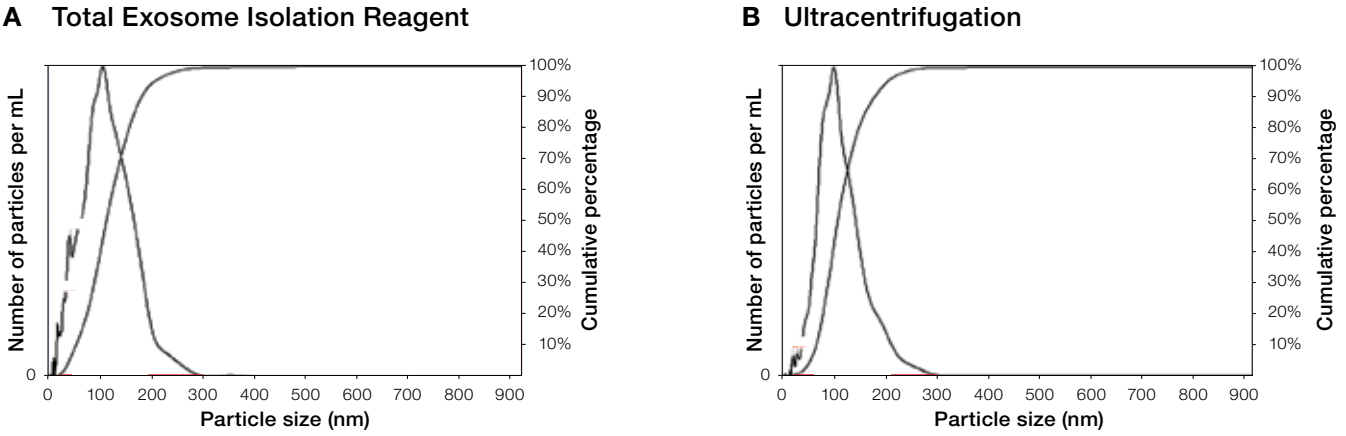

Figure 1. Analysis of exosomes recovered from HeLa cell media. (A) Exosomes recovered with the Invitrogen™ Total Exosome Isolation Reagent (from cell culture media) have a yield and size distribution comparable to **(B)** exosomes isolated following a traditional ultracentrifugation protocol with a sucrose gradient. Profiles as analyzed on a NanoSight™ LM10 instrument show all particles to be smaller than 300 nm; most are about 50–150 nm in size.

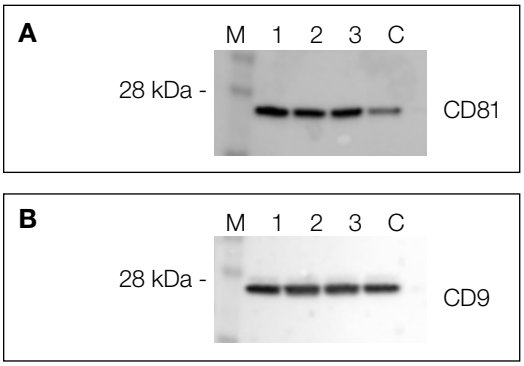

Figure 2. Analysis of exosomal markers CD81 and CD9. Exosomes were preenriched from SW480 cell cultures by ultracentrifugation. Immunoaffinity-based Dynabeads magnetic separation was used to further purify **(A)** CD81-positive or **(B)** CD9-positive exosomes from 15 µL preenriched samples (lanes 1–3). The isolated subpopulations were subjected to western blot analysis with antibodies against CD81 and CD9. M: molecular weight marker. C: Control with 7.5 µL preenriched exosomes without further isolation.

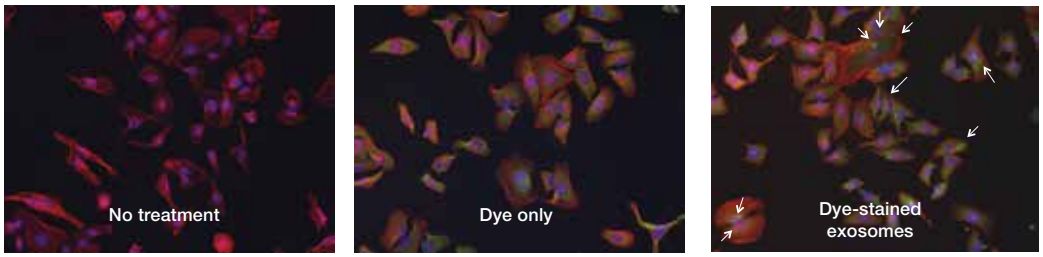

Figure 3. Uptake by HeLa cells of labeled exosomes. An Invitrogen™ FLoid™ Cell Imaging Station was used. Red: Invitrogen™ Alexa Fluor™ 594 phalloidin; blue: DAPI; green: Invitrogen™ SYTO™ RNASelect™ stain.

Find out more at **thermofisher.com/exosomes**

ThermoFisher SCIENTIFIC