ExoPure™ Reagent (Overall Exosome Isolation, cell media)

(Cat# *M1002-25, -50; Store at 4°C) *(*Not available for sale in USA)*

I. Introduction:

Exosomes are small endosome derived lipid nanoparticles (50-120 nm) actively secreted by exocytosis by most living cells. Exosome release occurs either constitutively or upon induction, under both normal and pathological conditions, in a dynamic, regulated and functionally relevant manner. Both the amount and molecular composition of the released exosomes depend on the state of a parent cell. Exosomes have been isolated from diverse cell lines (hematopoietic cells, tumor lines, primary cultures, and virus infected cells) as well as from biological fluids in particular blood (e.g. serum and plasma from cancer patients) and other body fluids (broncho alveolar lavage fluid, pleural effusions, synovial fluid, urine, amniotic fluid, semen, saliva etc). Exosomes have pleiotropic physiological and pathological functions and an emerging role in diverse pathological conditions such as cancer, infectious and neurodegenerative diseases.

ExoPure™ is a fast and convenient one-step method of exosome isolation from biofluids (i.e. plasma, serum and urine) and from cell culture media. Isolation with ExoPure™ is based on chemical precipitation. Samples are incubated with ExoPure™ solution on ice, so that exosomes will precipitate following centrifugation. The obtained pellet can be resuspended in 1X PBS or deionized water. The protocol is user friendly, timesaving (around 1 hour), and does not require capital laboratory equipment.

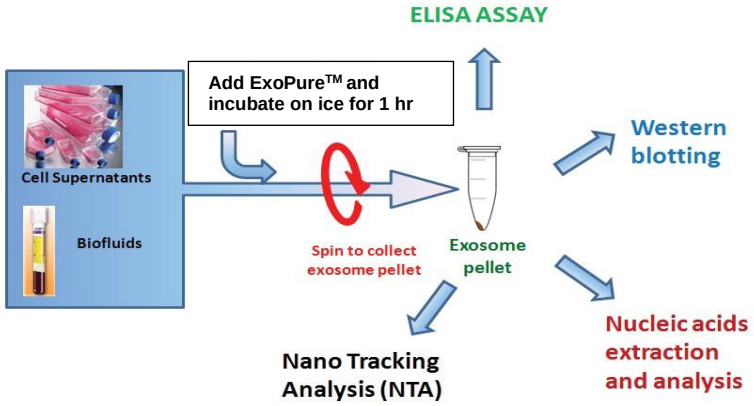


Figure 1. Exosome isolation Reagent.

II. Application:

- Single step isolation of exosomes from cell culture supernatants.
- ExoPure™ is able to isolate the overall exosome population from small volumes of sample (as low as 100 µl of plasma).
- Isolated exosomes are suitable for a wide range of analyses, such as nano tracking analysis (NTA), protein profiling by using different techniques (western blotting, ELISA, FACS), nucleic acid extraction and profiling of mRNA or small RNA markers.
- Easy to store and ships at 4°C.

III. Sample Type:

- Cell culture supernatants.

IV. Kit Contents: (exosome Isolation from cell culture supernatants):

Components	*M1002-25	*M1002-50	Part Number
	25 ml	50 ml	
ExoPure™ Reagent (cell media)	1 bottle (25 ml, 25 reactions)	2 bottles (2 X 25 ml, 50 reactions)	M1002-XX

V. User Supplied Reagents and Equipment:

- Single-use and/or pipettes with disposable tips 2-100 µl

VI. Shipment and Storage:

- ExoPure™ reagent is shipped at room temp and is recommend to store at 4°C for up to 12 months, if unopened. DO NOT FREEZE!

VII. Reagent Preparation and Storage Conditions:

- ExoPure™ reagent should be stored at 4°C.

VIII. ExoPure™ (exosome Isolation from cell media) Assay Protocol:

Volume suggested:

Fluid	Minimum volume required	Volume suggested
Cell medium	1 ml	1 ml- 5 ml

1. Cell medium sample preparation: Preclear cell supernatant to eliminate cell debris and macrovesicles by 3 centrifugation steps.

a) 10 min at 300g (save supernatant, discard the pellet).

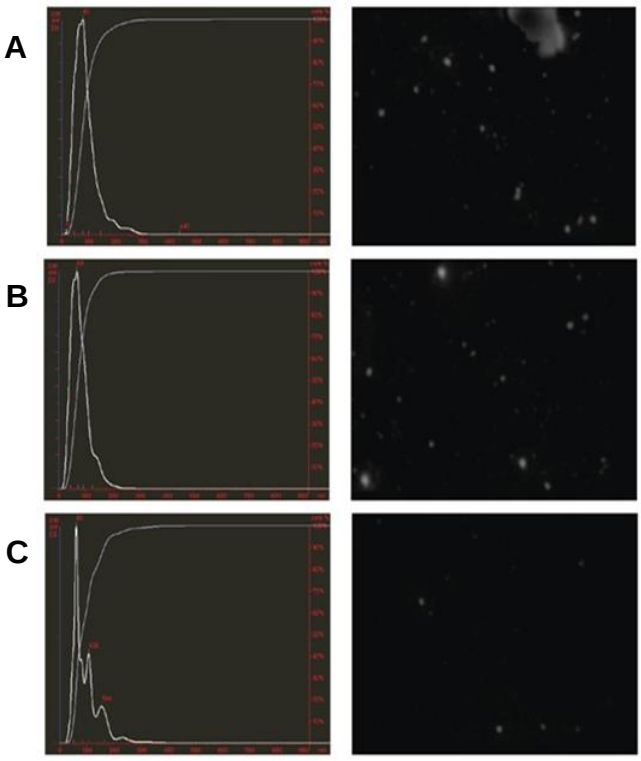
b) 20 min at 1200g (save supernatant, discard the pellet).

c) 30 min at 10,000g (save supernatant, discard the pellet).

2. Exosome isolation:

a) Add ExoPure™ solution to your sample in ratio 1/1 ((i.e 1 ml of cell medium + 1 ml of ExoPure™)
b) Mix well by pipetting and inverting tube.
c) Incubate on ice for 1 hr.
d) Centrifuge 20 min at 10,000g (centrifuge can be performed at 4°C or at RT).
e) Discard the supernatant.
f) Centrifuge for 2 min at 1500g to eliminate entirely the supernatant.
g) Resuspend the pellet in 100 µl* of 1X PBS.
Resuspended exosomes can be used for analysis or stored at -20°C. * Volume of resuspension can be defined by the user on the base of downstream analysis. Final exosome yield can be dependent on the cell line used. Different cell lines produce different quantity of exosomes. If exosome yield is poor, increase the volume of medium, maintaining the ratio with ExoPure™ 1/1 (2 ml of cell medium + 2 ml of ExoPure™).

3. Sensitivity: NTA analysis of plasma sample pre and post ExoPure™ treatment shows isolation of almost the entire nanoparticle population. The number of nanoparticles isolated by ExoPureTM (8.67x10^11) closely corresponds to the estimated number present in whole plasma (8.92x10^11). Vice versa, the remaining plasma supernatant is almost completely depleted of nanoparticles (0.91x10^11). Isolated vesicles are suitable for multiple downstream analyses, such as protein profiling via western blotting (Figure 3) or ELISA (Figure 4) or miRNA marker profiling (Figure 5).



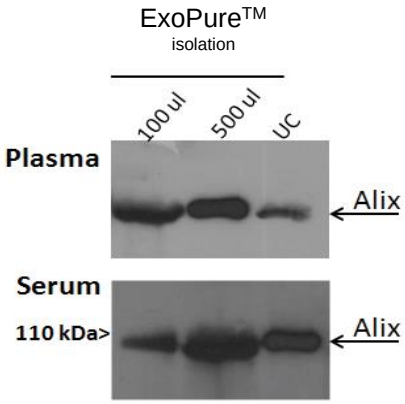


Figure 3. Detection of exosome marker Alix in protein lysates (30 µg) from exosomes via western blotting. Protein isolated using ExoPure™ from 100 µl and 500 µl of plasma/serum. Protein lysates (30 µg) from exosomes purified by ultracentrifugation (UC) was used as control.

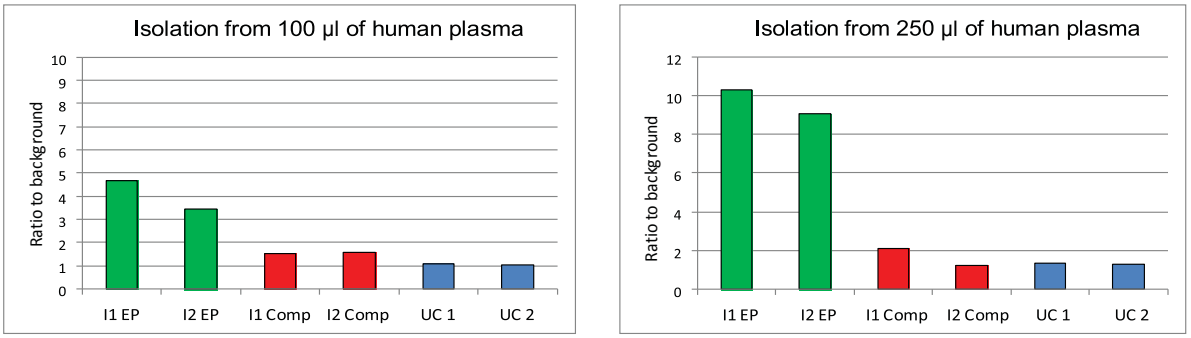


Figure 4. CD9 expression of plasma exosomes from two healthy individuals (100 µl and 250 µl) by ELISA. ExoPure™(EP) isolation reagent was compared to a competitor reagent (Comp) and exosomes purified by ultracentrifugation (UC).

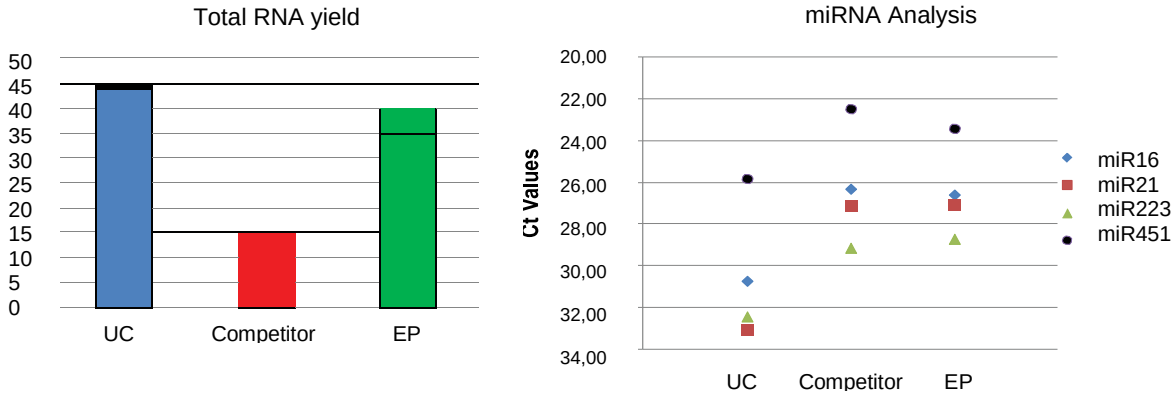


Figure 5. RNA extraction and profiling of 4 miRNAs from exosomes. Exosomes isolated by ExoPure™, (EP), a competitor reagent and ultracentrifugation (UC). 100 µl of plasma sample have been used.

ExoPure™ guarantees exosome isolation from complex biofluids, high performances and cheaper price than the competitor products.

Characteristics	ExoPure™	Competitor
Exosome isolation from cell supernatants	Yes	Yes, but pellet is hard to solubilize
Isolated exosome are intact and suitable for ELISA assay	Yes	Not suitable for ELISA quantification
Suitable for nucleic acids extraction and analysis	Yes	Yes
Price (for 25 ml of reagent)	●●	●●●

IX. Related Products:

Products/Catalog Number
ExoPure™ Reagent (Overall Exosome Isolation, biological fluids) # *M1001-5
ExoPure™ Reagent (Overall Exosome Isolation, biological fluids) # *M1001-10
ExoPure™ Reagent (Overall Exosome Isolation, biological fluids) # *M1001-20
ExoPure™ Reagent (Overall Exosome Isolation, cell media) # *M1002-25
ExoPure™ Reagent (Overall Exosome Isolation, cell media) # *M1002-50
ExoPure™ Reagent (Overall Exosome Isolation, urine) # *M1003-25
ExoPure™ Reagent (Overall Exosome Isolation, urine) # *M1003-50