EXO-Prep

One step Exosome isolation Reagent

Product Code: HBM-EXP-##

HansaBioMed Life Sciences

Pioneering the EV Field

About EXO-Prep

EXO-Prep is a fast and easy method of exosome isolation from biofluids and cell culture supernatants. Method is based on chemical precipitation. Samples are incubated with EXO-Prep solution in ice, then exosomes are separated by centrifugation and solubilized in PBS 1X or deionized water. Procedure is easy to perform, no time-consuming (around 1 hour) , does not require ultracentrifugation nor expensive laboratory equipment. Isolated exosomes are suitable for a wide range of analyses, such as NTA, protein profiling by using different techniques (western blotting, ELISA, FACS), nucleic acids extraction and profiling of mRNA or miRNA markers.

EXO-Prep available

Products	Volume	Catalog Number
EXO-Prep for exosome isolation from biofluids (plasma/serum)	5 ml	HBM-EXP-B5
EXO-Prep for exosome isolation from cell supernatants	25 m	HBM-EXP-C25
EXO-Prep for exosome isolation form urine	30 ml	HBM-EXP-U25

PROCEDURE FOR EXOSOME ISOLATION FROM PLASMA AND SERUM

Volume suggested

Fluid	Minimum volume required	Volume suggested
Plasma	100 µl	100 µl -250 µl
Serum	200 µl	250 µl - 500 µl

Sample preparation

Plasma and serum samples preparation

Prepare samples by 3 centrifugation steps at +4°C to eliminate red blood cells and cellular debris:

-10' at 300 g
-20' at 1 200 g
-30' at 10 000 g

Exosome isolation

- Add EXO-Prep solution to your sample in ratio 1/4 (i.e. 100 ul of plasma + 25 ul of EXO-Prep)
- Mix well by pipetting and inverting tube
- Incubate on ice for 1 hour
- Centrifuge 20 minutes at 10 000 g (centrifuge can be performed at 4°C or at RT)
- Discard the supernatant
- Centrifuge for 2 minutes at 1500 g to eliminate entirely the supernatant
- Resuspend the pellet in 100 ul* of PBS 1x
- 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.

PROCEDURE FOR EXOSOME ISOLATION FROM URINE

Volume suggested

Fluid	Minimum volume required	Volume suggested
Urine	5 ml	8 ml -20 ml

Sample preparation

Preclear urine as indicated:

- Centrifuge 10 min at 350 g at RT to eliminate cells and protein aggregates
- Save the supernatant.

Exosome isolation

- Add EXO-Prep solution to your sample in ratio 1/4 ((i.e 5 ml of urine + 1.2 ml of EXO-Prep)
- Mix well by pipetting and inverting tube
- Incubate on ice for 1 hour
- Centrifuge 20 minutes at 10000 g (centrifuge can be performed at 4°C or at RT)
- Discard the supernatant
- Centrifuge for 2 minutes at 1500 g to eliminate entirely the supernatant
- Resuspend the pellet in 100 ul* of PBS 1x
- 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.

PROCEDURE FOR EXOSOME ISOLATION FROM CELL CULTURE MEDIA

Volume suggested

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

Sample preparation

Cell medium preparation

Preclear cell supernatant to eliminate cell debris and macrovesicles by 3 centrifugation steps at +4°C

- I. 10' at 300xg (save supernatant, discard the pellet)
- II. 20' at 1200xg (save supernatant, discard the pellet)
- III. 30' at 10 000xg (save supernatant, discard the pellet)

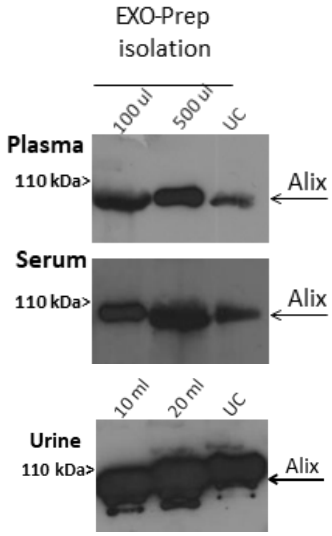
Exosome isolation

- Add EXO-Prep solution to your sample in ratio 1/1 ((i.e 1 ml of cell medium + 1 ml of EXO-Prep)
- Mix well by pipetting and inverting tube
- Incubate on ice for 1 hour
- Centrifuge 20 minutes at 10000 g (centrifuge can be performed at 4°C or at RT)
- Discard the supernatant
- Centrifuge for 2 minutes at 1500 g to eliminate entirely the supernatant
- Resuspend the pellet in 100 ul* of PBS 1x
- 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, mantaining the ratio with EXO-Prep 1/1 (2 ml of cell medium + 2 ml of EXO-Prep.

DATA ANALYSIS



Western blotting plasma/serum

A complex biofluid as **plasma** presents a high contents of proteins that coprecipitate with exosomes. We recommend to resuspend the pellet in 100 ul of PBS 1X and to quantify the protein contents via BCA or Bradford assay. For WB analysis we suggest to load on the gel more than 30 ug of total protein contents. If **serum** is used the entire pellet can be resuspended in an appropriate volume of PBS and loaded on the gel (refer to example below).

Western blotting Urine/conditioned media.

For WB analysis the entire pellet can be solubilized in the appropriate volume of PBS 1X and used for analysis (refer to the example and conditions described for urine).

ELISA assay

After exosome isolation resuspend pellet in 100 ul of PBS 1X and load the entire amount in a well of an ELISA* plate.

*HBM-precoated immunoplate or HBM Quantification kits are suggested for exosome capture and analysis of exosomal protein markers (see Related Products, page 9)

Nucleic acids extraction

Exosome pellet can be directly lysed with lysis buffers for nucleic acids extraction. If RNA extraction is performed by using Trizol or similar organic reagents we suggest to resuspend exosomes in PBS, then to add Trizol into the mixture and to proceed with RNA purification