Datasheet

EV Precipitation Solution (Urine, Blood, Cell Culture)

This product is for research use only.
It is highly recommended to read this users guide in its entirety prior to using this product.
Do not use this kit or its components beyond the indicated expiration date.

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PRODUCT DESCRIPTION

Product overview

Method is based on chemical precipitation. Samples are incubated with EV Precipitation 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.

EV Precipitation Solution advantages

Protocol easy to perform
- No time consuming
- No ultracentrifugation required
- Isolate exosomes from cell culture supernatants or biofluids
- Able to isolate the overall exosome population in a sample
- Isolate exosomes from a small volume of sample (plasma/serum 100 ul)
- For complex biofluids as plasma, no trombin pretreatment is required
- Isolated exosomes are intact and suitable for different downstream analyses

About Exosomes

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 amount and molecular composition of released exosomes depend on the state of a parent cell. Exosomes have pleiotropic physiological and pathological functions and an emerging role in diverse pathological conditions such as cancer, infectious and neurodegenerative diseases.

EV Precipitation Solution available:

Products	Volume	Catalog Number
EV Precipitation Solution (Blood)	5 ml	NBP3-11765
EV Precipitation Solution (Cell Culture)	25 ml	NBP3-11767
EV Precipitation Solution (Urine)	30 ml	NBP3-11766

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 to eliminate red blood cells and cellular debris:

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

Exosome isolation

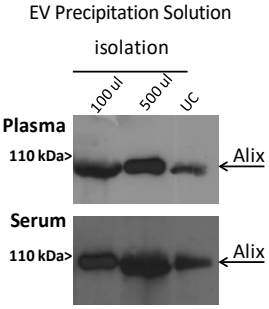
- Add solution to your sample in ratio 1/4 (i.e. 100 ul of plasma + 25 ul of EV Precipitation Solution)
- Mix well by pipetting and inverting tube
- Incubate on ice for 1 hour
- Centrifuge 20 minutes at 10000 g (centrifuge can be performed at 4°C or at RT)
- Discard the supernatant
- Centrifuge for 2 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.

DATA ANALYSIS

Western blotting

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).



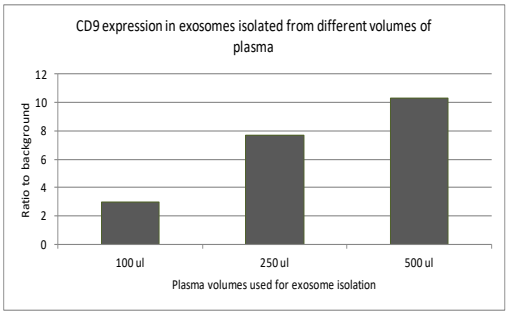
Plasma

Exosomes isolated from plasma were resuspended in 100 ul of PBS and protein contents quantified by BCA assay. 60 ug of total protein contents were loaded with Laemmli Sample buffer 5X on acrylamide gel. WB performed using anti-ALIX antibody. Ultracentrifuged exosomes used as control (UC)

Serum

Exosomes isolated from serum were resuspended in 24 ul of PBS 1X, and the entire amount was loaded with 6 ul of Laemmli Sample buffer 5X on acrylamide gel.

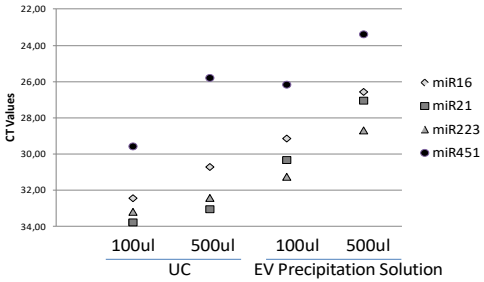
ELISA assay



Plasma and serum:

After exosome isolation resuspend pellet in 100 ul of PBS 1X and load the entire amount in a well of an ELISA* plate. Example reports CD9 expression in isolated exosomes from different volumes of plasma.

Nucleic Acid extraction



Plasma and serum:

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.

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

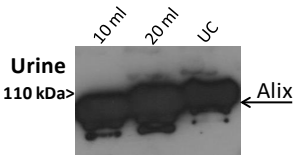
- Add solution to your sample in ratio 1/4 ((i.e 5 ml of urine + 1.2 ml of EV Precipitation Solution)
- Mix well by pipetting and inverting tube
- Incubate on ice for 1 hour
- Centrifuge 20 minutes at 10000 g (centrifuge can be performed at 4°C or at RT)
- Discard the supernatant
- Centrifuge for 2 minutes 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.

DATA ANALYSIS

Western blotting

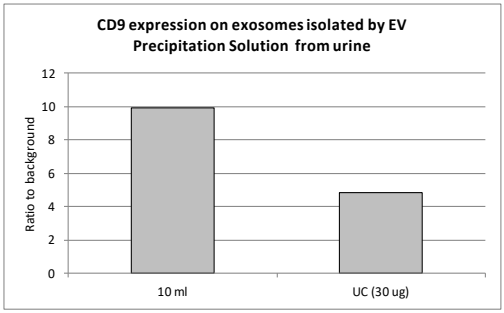
For WB the entire pellet can be solubilized in the appropriate volume of PBS 1X and used for analysis (refer to the example below).



Urine

Exosomes isolated from 10 ml and 20 ml of urine were resuspended in 24 ul of PBS 1X, and the entire amount was loaded with 6 ul of Laemmli Sample buffer 5X on acrylamide gel. Ultracentrifuged exosomes (30 ug) were used as control. Western blotting performed using anti-Alix.

ELISA assay



Urine

After exosome isolation resuspend pellet in 100 ul of PBS 1X and load the entire amount in a well of an ELISA* plate. Example reports CD9 expression in isolated exosomes from 10 ml of urine. 30 ug of purified exosomes via ultracentrifuge (UC) were used as control

Nucleic Acid 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.

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

- I. 10' at 300xg (save supernatant, discard the pellet)
- II. 20' at 1200xg (save supernatant, discard the pellet)
- III. 30' at 10000xg (save supernatant, discard the pellet)

Exosome isolation

- Add solution to your sample in ratio 1/1 ((i.e 1 ml of cell medium + 1 ml of EV Precipitation Solution)
- 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 EV Precipitation Solution 1/1 (2 ml of cell medium + 2 ml of EV Precipitation Solution).

DATA ANALYSIS

Western blotting

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.

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

RELATED PRODUCTS

Products	Quantity	Catalog Number
Human Exosome (Plasma) ELISA Kit (Colorimetric)	Ready to Use Kit	NBP3-11770
Human Exosome (Cell Media) ELISA Kit (Colorimetric)	Ready to Use Kit	NBP3-11772
Human Tumor-derived Exosome ELISA Kit (Colorimetric)	Ready to Use Kit	NBP3-11773
CD9 Immunobeads for Exosome Isolation	10 or 20 reactions	NBP3-11768
CD63 Immunobeads for Exosome Isolation	10 or 20 reactions	NBP3-11769

Research Use

All products are sold for research or laboratory use only and are not intended to be administered to humans or used for medical diagnostics.