Instructions for Use
Life Science Kits & Assays

PME Exosome Enrichment Kit

Innuscreen GmbH

Order No.:
845-IR-0010050 50 reactions

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This documentation describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!

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1 Introduction

1.1 Intended use

The **PME Exosome Enrichment Kit** has been designed for fast (35-45 min) and simple operation, where exosomes can be separated from both small (>200 µl) and large (<10 ml) sample volumes. This method does not require any specialized equipment and results in high purity of a final product.

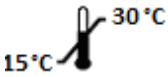
PME Exosome Enrichment Kit is based on a new technology, called PME – Polymer Mediated Enrichment. Adding of PME into any type of biological fluid (e.g. serum, plasma, urine, ascites, cell culture supernatant, fractions from EVs size separation by size exclusion chromatography (SEC)), efficiently immobilize exosomes within the net created by a special kind of polymer. Subsequently, the sample is centrifuged to form a pellet containing PME/exosomes complex. Obtained supernatant contains free proteins of the sample, including microRNAs-carrying proteins, like Ago2, HDL or LDL. The exosomes containing pellet can be resuspended in a dissolving buffer and used directly for downstream applications like microscopy, NanoSight, Western Blot, etc. The PME/exosomes pellet can be also lysed and exosomal microRNAs can be isolated with the use of **innuPREP cell-free microRNA Kit**. microRNAs from the exosome-depleted supernatant, as well as from the starting material can be isolated by the i**nnuPREP cell-free microRNA Kit** as well.

The kit is intended for use by professional users. The kit has been designed to be used for a wide range of different downstream applications, like amplification reactions and further analytical procedures.

CONSULT INSTRUCTION FOR USE
This package insert must be read carefully before use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.2 Notes on the use of this manual and the kit

For easy reference and orientation, the manual and labels use the following warning and information symbols as well as the shown methodology:

Symbol	Information
	**REF** Catalogue number.
	**Content** Contains sufficient reagents for <N> reactions.
	**Storage conditions** Store at room temperature or shown conditions respectively.
	**Consult instructions for use** This information must be observed to avoid improper use of the kit and the kit components.
	**Expiry date**
	**Lot number** The number of the kit charge.
	**Manufactured by** Contact information of manufacturer.
	**For single use only** Do not use components for a second time.
	Note / Attention Observe the notes marked in this way to ensure correct function of the kit and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → “Notes on the use of this manual and the kit" p. 3).
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully before use to guarantee your own safety and a trouble-free operation.
Follow all the safety instructions explained in the manual, as well as all messages and information, which are shown.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

FOR SINGLE USE ONLY!
This kit is made for single use only!

ATTENTION!
Don’t eat or drink components of the kit!
The kit shall only be handled by educated personal in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles to avoid any injuries. IST Innuscreen GmbH has not tested the liquid waste generated during using the kit for potential residual infectious components. This case is highly unlikely but cannot be excluded completely. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

Please observe the federal, state and local safety and environmental regulations. Follow the usual precautions for applications using extracted nucleic acids. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

ATTENTION!
Do not add bleach or acidic components to the waste after sample preparation!

NOTE
Emergency medical information in English and German can be obtained 24 hours a day from:
Poison Information Center, Freiburg / Germany
Phone: +49 (0)761 19 240.

For more information on GHS classification and the Safety Data Sheet (SDS) please sds.innu@ist-ag.com.

3 Storage conditions

The kit is shipped at ambient temperature.
Upon arrival, store Enrichment Reagent VCR-1 at 4 °C to 8 °C.
All other components of the **PME Exosome Enrichment Kit** should be stored dry at room temperature (15 °C to 30 °C). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box.

Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions dissolve these precipitates by careful warming.

4 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This kit was produced in an ISO 13485 certified facility.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the **PME Exosome Enrichment Kit** or other products, please do not hesitate to contact us. For technical support or further information in Germany please contact info.innu@ist-ag.com. For other countries please contact your local distributor.

5 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Product specifications” p. 8). Since the performance characteristics of IST Innuscreen GmbH kits have just been validated for the application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits using other protocols than those described below. IST Innuscreen GmbH kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA ´88 regulations in the U.S. or equivalent regulations required in other countries.

All products sold by IST Innuscreen GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE
The kit is for research use only!

6 Kit components

6.1 Included kit components

	Σ 50
REF	845-IR-0010050
Enrichment Reagent VCR-1	5 x 1.2 ml
Enrichment Reagent VCR-2	32 ml
10x Dissolving Buffer P	2 x 2 ml
Manual	1

6.2 Components not included in the kit

- 1.5 ml, 2.0 ml or 15 ml reaction tubes
- ddH_2O for dissolving the 10x Dissolving Buffer P
- RIPA/Triton Lysis Buffers

7 Product specifications

1. Starting material:

- Serum, plasma, urine, ascites, cell culture supernatants or mediums, SEC fractions and other cell-free bio-fluids from 0.2 ml up to 10 ml
  Cell cultures (max. 5 x 10^6 cells)

NOTE
For urine samples we recommend volumes >1 ml
Avoid freezing and thawing of starting material

2. Time for isolation:

- Approximately 35-45 minutes

8 Initial steps before starting

- Dissolving Buffer P

Prepare a multiplication of 1 volume of 10 x Dissolving Buffer P and 9 volumes of ddH_2O per every planned sample, e.g. if sample pellet is going to be dissolved in 200 µl of 1 x Dissolving Buffer P, mix 20 µl of 10 x Dissolving Buffer P with 180 µl of ddH_2O.

NOTE
Instead the usage of Dissolving Puffer P it is also possible to use RIPA+, Triton+ or your own established Dissolving Buffer. Store at 4°C up to 1 month. Protect from light.

Recipe for Triton Lysis Buffer:

Component	1 x	2 x
NaCl	150 mM	300 mM
Triton X-100 (or NP-40)	1%	2%
Tris pH 7.6	50 mM	100 mM

Recipe for RIPA Lysis Buffer:

Component	1 x	2 x
NaCl	150 mM	300 mM
IGEPAL CA -630	1%	2%
Natrium-Deoxycholat	0,5% (w/v)	1% (w/v)
SDS	0,1% (w/v)	0,2% (w/v)
Tris pH 7.6	50 mM	100 mM
Protease Inhibitors Cocktail	1x	2x

- RIPA+ / Triton+

  Prepare a multiplication of 1 volume of 10 x Dissolving Buffer P, 5 volumes of 2 x RIPA/Triton Lysis Buffer and 4 volumes of ddH2O, e.g. if sample pellet is going to be dissolved in 200 µl of RIPA+/Triton+, mix 20 µl of 10 x Dissolving Buffer P with 100 µl of 2 x RIPA /Triton Lysis Buffer and 80 µl of ddH2O.

- Centrifugation steps should be carried out at room temperature.
- Ensure that the starting material is free of cells, cell debris and larger vesicles. For this purpose we recommend to perform 2-step centrifugation followed by ultrafiltration:

  1. Residual cells removal: centrifuge at 1,500 x g for 10 min (preferably in centrifuge with swing rotor). Transfer supernatant into new reaction tube.
  2. Cell debris removal: centrifuge at 16,000 x g for 10 min. Transfer supernatant into new reaction tube.
  3. Removal of apoptotic bodies and microvesicles: ultrafiltrate through 0.2 µm injection filter into new reaction tube.

9 Isolation of exosomes from cell-free bio-fluids up to 1.5 ml

1. Add **30 µl of Enrichment Reagent VCR-1** and the sample into a 1.5 ml or 2.0 ml reaction tube and vortex shortly.

2. Add **150 µl of Enrichment Reagent VCR-2** to the tube, mix shortly by vortexing. Incubate at room temperature for 1 minute.

3. Centrifuge at maximum speed (>14,000 x g (~16.000 rpm)) for 30 minutes, open the tube and remove the supernatant carefully as much as possible (supernatant may be kept for non-exosomal miRNAs isolation)

NOTE
Do not remove/disturb the pellet; it contains exosomes!

4. Spin down the tube for 10 seconds, open the lid and carefully remove the residuals of supernatant.

5. Add minimum of 100 µl of **1x Dissolving Buffer P** (optionally your own dissolving buffer) and dissolve the pellet by piercing it by the end of the tip and by pipetting up and down several times. Avoid thereby the formation of air bubbles! Leave your samples for 10 min on ice to allow complete dissolving. Alternatively, proceed the undissolved pellet with the **innuPREP cell-free microRNA Kit**.

NOTE
Type and volume of buffers used to dissolve/lyse the exosome pellet depend on the downstream application; sample type and exosome/protein concentration and must be established experimentally. Dissolving Buffers simply resolve the PME structure to release intact exosomes. Lysis buffers RIPA+ and Triton+ dissolve PME pellet, lyse exosomes, release and protect proteins.

10 Isolation of exosomes from cell-free bio-fluids from 1.5 ml up to 10 ml

1. Add **100 µl of Enrichment Reagent VCR-1** and the sample into a 15 ml reaction tube and vortex shortly.
2. Add **600 µl of Enrichment Reagent VCR-2** to the 15 ml reaction tube, mix shortly by vortexing. Incubate at room temperature for 10 minutes.
3. Centrifuge at maximum speed (>14,000 x g (~16,000 rpm)) for 30 minutes, open the tube and remove the supernatant carefully as much as possible (supernatant may be kept for non-exosomal miRNAs isolation).

NOTE

Do not remove/disturb the pellet; it contains exosomes!

If the centrifuge reaching the maximum speed for higher sample volumes is not available, centrifuge your sample at least at 4,200 x g (~5,000 rpm). Higher speed increase efficiency of exosomes separation. Alternatively, aliquot the sample from point 2. into 1.5/2.0 ml tubes and centrifuge at >14.000 x g

4. Spin down the tube for 10 seconds, open the lid and carefully remove the residuals of supernatant.
5. Add minimum of 100 µl of **1x Dissolving Buffer P** (optionally your own dissolving buffer) and dissolve the pellet by piercing it by the end of the tip and by pipetting up and down several times. Avoid thereby the formation of air bubbles! Leave your samples for 10 min on ice to allow complete dissolving. Alternatively, proceed the undissolved pellet with the **innuPREP cell-free microRNA Kit**.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
No pellet after first centrifugation step	
Pellet overlooked	Pellet obtained from some kinds of samples might be transparent and colourless. Make sure to not overlook the pellet.
Insufficient addition of VCR-1 or VCR-2	Make sure that both VCR-1 and VCR-2 are added to the reaction tube. Make sure that the right volume of VCR-1 and VCR-2 are added.
Insufficient centrifugation	Make sure that centrifugation steps are carried out as describe in the manual. Otherwise repeat centrifugation.
Discarding of pellet	Ensure that the pellet is not discarded during removing the supernatant. In some cases, the pellet cannot be seen until the supernatant is removed completely.
Pellet is difficult to dissolve	
Too much addition of VCR-1 or VCR-2	Make sure that both VCR-1 and VCR-2 are added as described in protocol.
Not enough of dissolving buffer added to pellet	We recommend adding minimum 100 µl of dissolving buffer. Depending on the sample properties adding higher volumes of dissolving buffer might be necessary.
Pipette tip is clogged while dissolving the pellet	Cut the slide edge of pipette tip and try to transfer the pellet as much as possible.

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