EXO-NET® Pan-Exosome Capture

REF 40031

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Intended Use

Research Use Only. Not for use in diagnostic or therapeutic procedures.

EXO-NET® Pan-Exosome Capture is intended for use in the isolation and downstream analysis of extracellular vesicles (EVs, including exosomes) from biological samples for research applications. The product may not be resold, modified for resale, or used to manufacture commercial products without prior written approval and/or commercial licensing from INOVIQ Ltd.

Product Description

Exosomes are small extracellular vesicles released by most cell types that function as intercellular messengers, delivering their cargo of effector or signalling macromolecules between specific cells.[1]

EXO-NET® Pan-Exosome Capture is a proprietary immunoaffinity magnetic bead capture technology that uses a multi-antibody matrix coated on paramagnetic nanobeads to capture exosomes from human biofluids and cell-conditioned medium. EXO-NET® Pan-Exosome Capture captures nanovesicles based on the expression of specific antigenic epitopes on the surface of vesicles.

EXO-NET® Pan-Exosome Capture that allows for the rapid isolation of small extracellular vesicles from multiple cells and designed for use with serum, plasma, urine, and cell culture.

Applications

EXO-NET® Pan-Exosome Capture is a research use only product. EXO-NET® Pan-Exosome Capture isolated EVs can be used for the downstream analysis of DNA, RNA, proteins, and lipids using, for example, qRT-PCR, digital PCR, RNAseq, Mass Spectrometry, Western blot and ELISA.

Product Contents

Each vial of EXO-NET® Pan-Exosome Capture contains 1mL[2] of functionalised capture beads for processing plasma, serum, urine, saliva, or cell culture-derived exosomes.

Storage, Stability and Handling

Store the vial at 2-8°C. Return to storage conditions immediately after use.
DO NOT FREEZE.
DO NOT VORTEX.

[1] Zhang Y, Liu Y and Tang WH (2019) Exosomes: biogenesis, biologic function and clinical potential. Cell Biosci. https://doi.org/10.1186/s13578-019-0282-2
[2] Sufficient for approximately 66 isolations using 15µL per 200 µL of plasma

Required Materials and Equipment

1X Phosphate buffered saline (PBS) Filter Sterilized before use.
Magnetic tube stand (Invitrogen MagnaRack Magnetic Separation Rack, or similar)
Tube rack
Microcentrifuge tubes
Micro pipettor
Sterile Pipette tips

Protocol

The volume of EXO-NET® Pan-Exosome Capture beads may be adjusted according to target abundance and application. It is advisable to run an initial sample volume titration study to determine the optimal amount of beads and sample needed for your downstream requirements.
The following protocol provides the basic steps required for using EXO-NET® Pan-Exosome Capture:

1. Defrost human plasma by incubating at RT for ~ 15 min.
2. Warm the EXO-NET beads to RT.
3. Label 1.5 mL microfuge tubes for each sample and place them in a nonmagnetic rack.
4. Add 200 µL of plasma into 1.5 mL microfuge tubes.
5. Centrifuge plasma for 5 min at 10000 x g, RT.
6. Transfer the supernatant to a new tube.
7. Resuspend EXO-NET beads by gently pipetting 10 times to disperse EXO-NET beads.
8. Once homogenous, add 15 µL of EXO-NET beads to each tubes containing plasma.
9. Cap the tubes. Thoroughly mix the beads and plasma cocktail by flicking the tube 10 times. Avoid forming bubbles.
10. Incubate the mixture for 15 min at RT.
11. Place the tubes in the magnetic rack, for at least 5 min or until the liquid is clear.
12. Remove the supernatant carefully using a p1000 pipette. Guide the pipette tip towards the clear side of the tube to avoid bead loss. Discard the supernatant in the appropriate waste stream.
13. Resuspend beads in 1000 µL filtered DPBS to wash. Gently target the bead pellet while dispensing the buffer to bring the beads in suspension. Do not invert or vortex the tubes.
14. Place the tubes in the magnetic rack, for 5 min or until the liquid is clear.
15. Remove the supernatant carefully using a p1000 pipette. Guide the pipette tip towards the clear side of the tube to avoid bead loss. Discard the supernatant in the appropriate waste stream.
16. Perform DPBS wash two additional times. Remove the maximum volume of wash solution after each wash.
17. The final pellet can then be lysed as required for the desired downstream application. In the case of Western Blots, please refer to our Application Note[3].

[3] Western Blot Analysis of EXO-NET® captured Extracellular Vesicles Application Note (https://www.inoviq.com/site/products/exo-net-pan-exosome/resources)

18. Following lysis, place the tubes in the magnetic rack, for at least 5 min or until the liquid is clear.
19. Remove the supernatant carefully using p200 into a new tube. Guide the pipette tip towards the clear side of the tube to avoid taking beads.
20. Proceed with desired downstream application.

Notes:

The volume of input can be increased according to the requirements of the downstream assay system. For example, use 0.5 mL plasma and 30 µL of EXO-NET® Pan-Exosome Capture. A low concentration of EV in the starting material does not require more beads or a longer incubation time. Gentle mixing by flicking the tube may improve recovery.

Warnings and Precautions

1. EXO-NET® Pan-Exosome Capture beads contain the preservative ProClin™ 300 at a concentration of 0.05% (w/w). It contains the active ingredients 2-methyl-4-isothiazolin-3-one and 5-chloro-2-methyl-4-isothiazolin-3-one. Wear appropriate personal protective equipment when handling this product, as exposure may cause irritation to the skin, eyes, mucous membranes, and upper respiratory tract.
2. The concentration of ProClin™ 300 in this product does not meet the OSHA criteria for a hazardous substance.
3. Biofluids, and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents or specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amount of soap and water. Seek medical advice.
4. Consult local and/or state authorities to determine the recommended method of disposal.

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