

# INSTRUCTIONS FOR USE

## EXO-NET® PAN-EXOSOME CAPTURE

For Research Use Only.

**REF** 40031, 40033, 40036



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## INTENDED USE

EXO-NET Pan-Exosome Capture is intended for use for the isolation and downstream analysis of human extracellular vesicles (EVs, including exosomes) from different human biofluids 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<sup>1</sup>.

EXO-NET 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 isolates nanovesicles based on the expression of specific antigenic epitopes on the surface of vesicles.

EXO-NET that allows for the rapid isolation of small extracellular vesicles from multiple cells and designed for use with serum, plasma, urine, saliva and cell-conditioned medium.

## APPLICATIONS

EXO-NET is a research use only product. EXO-NET 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 contains functionalised capture beads for processing plasma, serum, urine, saliva or cell culture-derived exosomes.

Cat No.	Product Name	Isolations*	Size
40031	EXO-NET® Pan-Exosome Capture	60	1.0mL
40033	EXO-NET® Pan-Exosome Capture	30	0.5mL
40036	EXO-NET® Pan-Exosome Capture	15	0.25mL

\* Isolations based on 15µl of EXO-NET® per 200µl plasma

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## STORAGE, STABILITY AND HANDLING

Store the vial at 2-8°C. Return to storage conditions immediately after use.

DO NOT FREEZE.

**DO NOT VORTEX.**

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## 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.

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## PROTOCOL

The volume of EXO-NET beads may be adjusted according to biofluid 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:

1. Defrost human plasma at RT for ~ 15 min.
2. Aliquot the required volume of EXO-NET beads into a microfuge tube and keep at 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 each 1.5 mL microfuge tube.
5. Centrifuge plasma for 5 min at 10,000 x g at RT.
6. Transfer the supernatants to new 1.5 mL microfuge tubes.
7. Resuspend EXO-NET beads by gently pipetting 10 times immediately prior to adding to sample tubes
8. Add 15 µL of EXO-NET beads to each sample tube.
9. Cap the tubes. Mix the beads and plasma by gently 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. Carefully remove the supernatant 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. Carefully remove the supernatant 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 INOVIQ’s Application Note<sup>2</sup>.
18. Following lysis, place the tubes in the magnetic rack for at least 5 min or until the liquid is clear.
19. Leave the tubes in the magnetic rack and carefully transfer the lysate 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 sample can be increased according to the requirements of the downstream assay system. For example, use 0.5 mL plasma with 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.

## REFERENCES

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. Western Blot Application For EXO-NET® Captured Human Extracellular Vesicles (<https://www.inoviq.com/site/products/exo-net-pan-exosome/resources>)

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