EXO-NET® Pan-Exosome Capture

Instructions for Use of Products CS3419A01, CS3419A15

1. Description

EXO-NET® Pan-Exosome Capture is an immunoaffinity-based magnetic bead technology designed to isolate extracellular vesicles (EVs), including exosomes, from human biofluids such as plasma, urine, and conditioned cell media. The beads are coated with INOVIQ's proprietary multi-antibody matrix targeting specific surface epitopes on EVs, enabling efficient and reproducible isolation. The streamlined workflow with EXO-NET enables rapid capture of EVs, with flexible capability of batch processing for high-throughput applications. By minimizing co-isolation of contaminants, EXO-NET delivers high purity EV preparations enriched for DNA, RNA and protein biomarkers, making it an optimal solution for both low-volume and rare samples. Captured vesicles can be used for downstream analysis of RNA, DNA, protein, and lipids using assays such as RT-qPCR, RNA-seq, digital PCR, Western blot, ELISA, and mass spectrometry. The EXO-NET approach allows more complete and consistent enrichment of EVs with less contamination by other biomolecules.

For Research Use Only. Not for use in diagnostic procedures.

2. Product Components and Storage Conditions

2.A. Products

Product	Size	Cat#	Contents/Notes
EXO-NET® Pan-Exosome Capture Reagent	**250µl**	**CS3419A01**	Sufficient for: (a) 15 isolations based on 15µl of EXO-NET per 200µl plasma, or 500µl urine or conditioned cell media (b) 7 isolations based on 30µl of EXO-NET per 500µl plasma, or 1ml urine or conditioned cell media
EXO-NET® Pan-Exosome Capture Reagent	**750µl**	**CS3419A15**	Sufficient for: (a) 48 isolations based on 15µl of EXO-NET per 200µl plasma, or 500µl urine or conditioned cell media, or

	(b) 24 isolations based on 30µl of EXO-NET per 500µl plasma, or 1ml urine or conditioned cell media

2.B. Component and Kit Storage Conditions

Store the EXO-NET® Pan-Exosome Capture Reagent at +2°C to +10°C. Do not freeze. Do not vortex. Return to storage conditions immediately after use.

3. Assay Considerations

- The volume of EXO-NET® beads may be adjusted based on the input biofluid. An initial bead volume titration is recommended to determine the optimal input and bead volume. Avoid vortexing the EXO-NET® beads. Gently flick or pipette-mix as required.
- The type of blood collection tube may affect downstream assays. EDTA is commonly used, while heparin is known to inhibit many molecular assays.
- Excessive centrifugation force can cause cell lysis, resulting in the release of additional vesicles. Use moderate centrifugation speeds during sample preparation to remove intact cells prior to EV isolation.

4. Materials to be Supplied by the User

- 1X DPBS, no calcium, no magnesium (e.g. Thermo Fisher Cat. #14190144), filtered with 0.2µm filter
- **Optional:** 1X TBS, optional, filtered with 0.2µm filter
  Note: 1X TBS, filtered is recommended for EXO-NET® workflows with subsequent purification using the Maxwell® RSC miRNA Plasma and Serum Kit (Promega Cat.# AS1680)
- High strength magnetic stand for tubes (e.g., Invitrogen DynaMag™-2 Magnet, or Permagen Magnetic Separation Rack)
- Tube rack (non-magnetic)
- Microcentrifuge tubes (1.5ml or 2ml)
  Note: Low protein-binding tubes are recommended (e.g., Eppendorf Protein LoBind Tubes Cat.# 0030108442)
- Pipettors, including P1000; and sterile, aerosol-resistant pipette tips
- Rotary mixer (required for urine and conditioned cell culture media samples, optional for plasma samples)

5. Protocols

The following protocols outline the general procedure for isolating extracellular vesicles (EV) using EXO-NET® Pan-Exosome Capture Reagent. Adjustments may be made and evaluated by the user based on sample volume and downstream application requirements.

5.A. Plasma Sample Protocol for EV Isolation

1. Warm EXO-NET® Pan Exosome Capture Reagent at room temperature for 15 minutes before use. Optional: while warming, place on a gentle rotary mixer to help break up any aggregates.
2. Label 1.5ml microcentrifuge tubes for each sample and place in a non-magnetic rack.
3. Add plasma sample to each microcentrifuge tube.
4. Resuspend EXO-NET® beads by pipetting gently 10 times with a P1000 pipettor immediately before use.
5. Add EXO-NET® beads to each plasma sample tube according to Table 1. Pipette to mix each sample as the beads are added.

Table 1. Volume of EXO-NET® beads for Plasma Samples

Plasma Sample Volume	Volume of EXO-NET® Pan-Exosome Capture beads to add
200µl	15µl
500µl	30µl

Note: The EXO-NET® bead volume can be adjusted to accommodate up to 1.0ml sample volume in a 1.5ml microcentrifuge tube. A titration experiment is recommended to determine the optimal amount of EXO-NET for different sample volumes.

6. Cap and gently flick the sample tube 10 times to mix. Avoid creating bubbles. Do not vortex.
7. Incubate plasma with EXO-NET® beads for 15 minutes at room temperature in a non-magnetic rack.
8. Place sample tubes into a high-strength magnetic rack until liquid is clear (approximately 5 minutes).
9. While sample tubes remain in the magnetic rack, carefully and slowly remove supernatant using a P1000 pipette. Keep the pipette tip on the side opposite the beads to avoid disturbing the EXO-NET® bead pellet.
10. Transfer sample tubes to a non-magnetic rack.
    Note: Leaving sample tubes in the high strength magnetic rack during the following wash steps may not wash the beads as effectively.
11. Resuspend beads in 1,000µl filtered 1X DPBS. Dispense buffer directly onto the EXO-NET® bead pellet.

12. Place sample tubes into the high-strength magnetic rack until liquid is clear (approximately 5 minutes).
13. While sample tubes remain in the magnetic rack, carefully and slowly remove supernatant using a P1000 pipette. Keep the pipette tip on the side opposite the beads to avoid disturbing the EXO-NET® bead pellet.
14. Repeat steps 11-13 two more times for a total of three washes with DPBS.
15. After the final wash, transfer sample tubes to a non-magnetic rack. EVs remain covalently bound to the EXO-NET® beads. Add the appropriate solution to resuspend the beads based on the intended downstream application:
    (a) For RNA isolation with Maxwell® RSC miRNA Plasma and Serum kit (Promega Cat.# AS1680), resuspend EVs bound to EXO-NET® beads in 200µl 1x TBS.
    (b) For protein isolation with MPSP beads (Promega Cat.# CS3325A03), resuspend EVs bound to EXO-NET® beads in 50µl lysis buffer (1 % SDS, 50 mM HEPES, pH 8.0).

Note:
(1) The choice of resuspension solution and volume may vary depending on the downstream assay(s) planned for the purified EVs.
(2) The EVs remain bound to the EXO-NET® beads. If downstream workflow includes a lysis step, the EXO-NET® beads may be removed *after* lysis by placing the sample tube into a high-strength magnetic rack and transferring the lysate supernatant to a new microcentrifuge tube or plate well.

5.B. Urine or Conditioned Cell Media Protocol for EV Isolation

1. Warm EXO-NET® Pan Exosome Capture Reagent at room temperature for 15 minutes before use. Optional: while warming, place on a gentle rotary mixer to help break up any aggregates.
2. Label 1.5ml microcentrifuge tubes for each sample and place them in a non-magnetic rack.
3. Add up to 1ml urine or conditioned cell media sample to each microcentrifuge tube as indicated in Table 2.
4. Resuspend EXO-NET® beads by pipetting gently 10 times with a P1000 pipettor immediately before use.
5. Add EXO-NET® beads to each sample tube according to Table 2. Pipette to mix each sample as the beads are added.

Table 2. Volume of EXO-NET® beads for Urine or Conditioned Cell Media Samples

Urine or Conditioned Cell Media Volume	Volume of EXO-NET® beads to add
500µl	15µl

1ml	30µl

Note: The EXO-NET® bead volume can be adjusted to accommodate up to 1.0ml sample volume in a 1.5ml microcentrifuge tube. A titration experiment is recommended to determine the optimal amount of EXO-NET for different sample volumes.

6. Cap and gently flick the tube 10 times to mix. Avoid creating bubbles. Do not vortex.
7. Incubate the urine or conditioned cell media sample with EXO-NET® beads for 30 minutes at room temperature on a rotary mixer.
8. Place sample tubes into a high-strength magnetic rack until liquid is clear (approximately 5 minutes).
9. While sample tubes remain in the magnetic rack, carefully and slowly remove supernatant using a P1000 pipette. Keep the pipette tip on the side opposite the beads to avoid disturbing the EXO-NET® bead pellet.
10. Urine and conditioned cell media sample tubes should **remain** on the magnetic rack for the following washing steps.
11. Add 1,000µl filtered 1X DPBS to wash the beads. Add buffer to the opposite side from the bead pellet.
12. Allow the liquid to clear (~5 minutes).
13. Carefully and slowly remove supernatant using a P1000 pipette. Keep the pipette tip on the side opposite the beads to avoid disturbing the EXO-NET® bead pellet.
14. Repeat steps 11-13 two more times for a total of three washes with DPBS.
15. After the final wash, transfer sample tubes to a non-magnetic rack. EVs remain covalently bound to the EXO-NET® beads. Add the appropriate solution to resuspend the beads based on the intended downstream application:
    (a) For RNA isolation with Maxwell® RSC miRNA Plasma and Serum kit (Promega Cat.# AS1680), resuspend EVs bound to EXO-NET® beads in 200µl 1x TBS.
    (b) For protein isolation with MPSP beads (Promega Cat.# CS3325A03), resuspend EVs bound to EXO-NET® beads in 50µl lysis buffer (1 % SDS, 50 mM HEPES, pH 8.0).

Note:

(1) The choice of resuspension solution and volume may vary depending on the downstream assay(s) planned for the purified EVs.

(2) The EVs remain bound to the EXO-NET® beads. If downstream workflow includes a lysis step, the EXO-NET® beads may be removed *after* lysis by placing the sample tube into a high-strength magnetic rack and transferring the lysate supernatant to a new microcentrifuge tube or plate well.

5.C. Protocol for Mass Spectrometric Sample Preparation using MPSP Magnetic Beads

Table 3. MPSP Magnetic Beads Product Information

Product	Size	Cat#
MPSP Magnetic Beads Slurry	**1 x 5 ml**	**CS3325A03**
Digestion Buffer	**1 x 25 ml**	**CS3325A05**

Materials to be Supplied by the User

- TCEP (ThermoFisher Scientific, Catalog No. 77720
- Chloroacetamide (Highest purity grade available)
- Trypsin/Lys-C mix (100 mg) (Promega Catalog No. V5072)
- 80% Ethanol (Highest purity grade available)
- 100% Acetonitrile (Highest purity grade available)
- Tube rack (non-magnetic)
- Magnetic stand
- Microcentrifuge tubes (1.5ml or 2ml)
  Note: Low protein-binding tubes are recommended especially if isolated extracellular vesicles are used for downstream proteomic analysis (e.g., Eppendorf Protein LoBind Tubes Cat.# 0030108442)
- Pipettors, including P1000; and sterile, aerosol-resistant pipette tips
- Shaker at room temperature

1. To the purified EV protein (step 15(b) from Protocol 5.A. or 5.B. above), add reducing agent (5 mM TCEP, 15 mM Chloroacetamide – final concentration) in a volume of 5 µl.
2. Incubate for 30 minutes at 37 ^{0}C.
3. Add MPSP magnetic beads (15 µl of a 25 mg/ml bead stock). This assumes a yield of 5-15 mg of protein from 0.2 ml of plasma. (Ratios from 1:2.5 - 1:50 are effective for capturing EV protein without significant variance.)
4. Add 280 µl of 100 % acetonitrile.
5. Incubate with shaking (1200 RPM) for 30 minutes at room temperature.
6. Place tubes or plate on a magnetic stand.
7. Remove supernatant.
8. Wash 2X with 80 % Ethanol (0.5 ml).
9. Wash 1X with 100 % Acetonitrile (0.5 ml).
10. Add 50 µl of digestion buffer (either MPSP digestion buffer or 50 mM HEPES, pH 8.0, 1 mM Calcium Chloride) containing 1.25 µg of trypsin in 5 µl (0.25 mg/mL).
    Note: The HEPES buffer is recommended if you are planning to perform a Peptide assay using the Pierce Quantitative Fluorometric assay or if using mass tags.
11. Incubate overnight at 37^{0}C with shaking (1200 RPM).

6. Troubleshooting

Symptoms	Causes and Comments
Low EV yield	Use fresh samples stored properly or freeze samples promptly to maintain high quality. Minimize the number of freeze/thaws. Long term storage of samples should be below -65°C.
	Incomplete resuspension of the EXO-NET® beads can lead to inconsistent amounts added to samples. Ensure that bead resuspension is thorough before use by pipetting with a P1000 pipettor. A rotary mixer may also be used.
	Not enough EXO-NET® beads were added to bind the available EVs in the sample. Use the recommended volume of EXO-NET® beads relative to input sample or optimize for other sample volumes.
	Avoid loss of EXO-NET® beads during wash steps. Use a high-strength magnetic rack (e.g., N52 magnets are recommended) and remove supernatant slowly. Point pipette tip away from the bead pellet when aspirating the supernatant.
	Do not vortex or centrifuge EXO-NET® Pan Exosome Capture reagent. Use a P1000 to mix and resuspend EXO-NET® beads.
	Transfer any liquid from the sample tube cap before magnetic separation in the wash step to avoid sample loss.
	Wider tubes (e.g., 15ml) increase the distance between the magnet and beads and therefore reduce the effective magnetic force strength. Tubes with a

	larger diameter may require longer binding time.
	Use low-protein binding tubes or low-retention tubes to avoid non-specific binding of EVs and EXO-NET® beads to the tube.
	EXO-NET® Pan Exosome Capture Reagent stored at the wrong temperature. EXO-NET should be stored at +2°C to +10°C between uses. Exposure to high heat or freezing temperatures may cause the EXO-NET® beads to lose activity, resulting in low recovery of EVs.
	Non-human EVs are not captured as efficiently as human EV. EXO-NET is designed for human samples.
	Different sample types may contain varying amounts of EVs, depending on factors such as cellular state, activity level and the duration of conditioned media exposure before collection.
Higher than expected EV yield	Excessive centrifugation force can cause cells lysis resulting in the release of additional vesicles. Use moderate centrifugation speeds during sample preparation to remove intact cells prior to extracellular vesicle isolation.
	Cell media with fetal bovine serum (FBS) will contain bovine EVs. Use exosome-depleted FBS.
	Ensure that wash solutions (e.g., DPBS, PBS, TBS) are sterile.
EXO-NET® beads are difficult to resuspend	Do not vortex. Use a rotary mixer in addition to pipette mixing with a P1000 pipettor to gently break up aggregates prior to use. Failure to do so may result in

	inconsistent amounts of EXO-NET® beads added to samples which may result in variable recovery of EVs.
High background in downstream assay	Ensure thorough washing of samples (after binding to EXO-NET® beads) with filtered DPBS.
	Failure to filter reagents before use with EXO-NET® beads may introduce contaminants.

7. Appendix

7.A. Safety and Handling

EXO-NET® beads contain the preservative 0.05% (w/w) ProClin™ 300. 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. Avoid contact with skin and eyes. Dispose of waste according to institutional biosafety procedures.

7.B. 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. Khanabdali R, Mandrekar M *et al*. High-throughput surface epitope immunoaffinity isolation of extracellular vesicles and downstream analysis. Biology Methods and Protocols 2024; 9: bpae032. DOI: 10.1093/biomethods/bpae032

3. Western Blot Application for EXO-NET® Captured Human Extracellular Vesicles. INOVIQ. Available at: Western Blot Application For EXO-NET® Captured Human Extracellular Vesicles

8. Related Products

Product	Size	Cat.#
Maxwell® RSC miRNA Plasma and Serum Kit	48 preps	AS1680
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500