ELISA PRODUCT INFORMATION & MANUAL

Exosome Capture (Biofluids, Cell Media) and RNA Extraction Kit

NBP2-49784

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.

Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Exosome Capture (Biofluids, Cell Media) and RNA Extraction Kit

For the isolation and efficient extraction of high-quality total RNA (miRNA and mRNAs) from the overall exosome population from human biofluids or cell culture media.
For research use only - not intended for diagnostic use.

Storage and Stability

All the reagents are shipped and stored at 4°C for up to 8 months, if unopened. Briefly centrifuge small vials prior to opening. Do not freeze.

Materials Supplied

Item	Quantity	Storage Condition
Pre-couple latex immunobeads	1 vial (20 reactions)	4°C
Bead Washing buffer (5X)	1 bottle (10 ml)	4°C
Lysis buffer	1 bottle (16 ml)	4°C
RNA Washing buffer (to add Ethanol 96%)	1 bottle (9 ml)	4°C
Elution buffer	1 vial (1 ml)	4°C
Columns	22 columns	4°C
RNase free elution tubes (1.5 ml)	22 tubes	4°C
Lyophilized Exosome Standard	1 vial (100 µg)	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Single-use and/or pipettes with disposable tips 2-100 µl
- Low-binding Eppendorf tubes (1.5 ml)
- Pipettes 1 ml and 5 ml for reagent preparation
- PBS
- Disposable pipetting reservoirs
- Ethanol 96%
- Chloroform or BCP (1-bromo-3-chloropropane)
- Sample concentrator (urine and cell culture supernatant samples)

Reagent and Sample Preparation

- Immunobeads can be stored at +4°C for up to 8 months.
- The RNase free columns and elution tubes must be stored at room temperature.
- All the opened buffers and diluted reagents including the bead washing buffer, RNA washing buffer, lysis buffer and the elution buffer should be stored at +4°C.

Bead Washing Buffer: Dilute bead washing buffer (5X) to 1X with deionized water. Ensure there is no crystal precipitate. Mix 5 ml of 5X Beads Washing Buffer with 20 ml deionized water for a final volume of 25 ml.

Δ Note: *If crystals are observed, dissolve them by warming up the concentrated 5X Washing Buffer bottle at 37°C before proceeding with the dilution.*

RNA Washing Buffer Solution: Add into RNA Washing Buffer bottle the volume of pure ethanol (96%) indicated on the bottle's label, to get an approximate final ethanol concentration of 70%.

Elution Buffer and Lysis Buffer are ready to use.

Sample Preparation

Plasma and Serum sample preparation:

Prepare plasma samples by 3 centrifugation steps to eliminate red blood cells and cellular debris.

a) 10 min at 300g (save supernatant; discard pellet).
b) 20 min at 1200g (save supernatant; discard pellet).
c) 30 min at 10,000g (save supernatant; discard pellet).

Urine sample preparation:

1. Centrifuge at 16,000g for 20 min at room temperature.
2. Filter by using 0.45 µm filter.
3. Concentrate urine samples by spin concentrator 15-20 times*****.

Cell supernatant sample preparation:

a) 10 min at 300g (save supernatant; discard pellet).
b) 20 min at 1600g (save supernatant; discard pellet).
c) 30 min at 10,000g (save supernatant; discard pellet).
d) Concentrate cell supernatant 10-20 fold in spin concentrator*****.

Δ Note: **The quantity of exosomes could vary between samples. A larger starting amount of sample should be used if the signal is weak.*

Assay Protocol

Exosome binding

Purified exosomes and the lyophilized standards do not require this binding step. If the samples are purified exosomes, skip to RNA extraction directly.

1. Place 0.1 ml up to 1 ml of sample into low-binding tubes (not provided in the kit). Volumes suggested: 0.1 ml up to 0.5 ml for small RNA analysis; 0.5 ml up to 1 ml for mRNA analysis.
2. Add 1X PBS to the sample to get a final volume of 1 ml. If you are using 1 ml of plasma, dilution is not necessary.
3. Add 10 µl of immunobeads.
4. Incubate sample-immunobead mixture overnight at 4°C in a rotator.
5. Centrifuge at room temperature (RT) for 10 min at 5,000 x g.
6. Discard the supernatant.
7. Wash the beads:
    a. Add 1 ml of Bead Washing Buffer.
    b. Resuspend up and down 10-15 times.
    c. Centrifuge at RT for 10 min at 5,000g.
    d. Remove the supernatant being careful not to disturb the pellet.
    e. Wash beads once again as indicated above.

RNA Extraction

Lysis

Purified Exosomes: Add 200 µl of Lysis buffer directly to lyophilized exosomes. Resuspend by pipetting and transfer to a fresh tube. Add 500 µl of Lysis buffer to reach a final volume of 700 µl. Incubate for 5 min at room temperature.

Unfractionated Samples: Add 700 µl of Lysis buffer directly on the bead pellet. Dissolve the pellet by pipetting up and down (beads must be totally dissolved). Incubate for 5 min at room temperature.

Extraction:

1. Add 70 µl of 1-Bromo-3-chloropropane (BCP) or 140 µl of pure Chloroform.
2. Shake 30 sec.
3. Incubate for 10 min at room temperature.
4. Incubate 1 min on ice and then centrifuge at 12,000 x g at 4°C for 10 min.

Δ Note: *Incubation on ice before centrifugation helps to reduce DNA contamination, which tends to remain in the interphase.*

5. Transfer the top phase (aqueous) to a fresh tube.
6. Add twice the volume of ethanol 96%; e.g., if the top phase volume is 400 µl, add 800 µl of ethanol 96%. Mix by gently inverting 4-5 times.

Purification:

1. Transfer half of the volume of the mixture into a spin column.
2. Spin at 14,000 x g for 30 sec.
3. Discard the flow-throw.
4. Add the remaining volume into the same spin column.
5. Spin at 14,000g for 30 sec. Discard the flow-throw.
6. Wash the column by adding 400 µl of RNA Washing buffer. Spin at 14,000 x g for 30 sec. Discard the flow-through. Perform the washing step twice more.
7. Spin for 5 additional min at 14,000 x g to eliminate the ethanol residues from the column. Discard the flow-throw.
8. Remove the tube and transfer the spin column into an elution tube.
9. Elute the column with 15 µl of Elution buffer. Incubate for 5 min at room temperature. Spin for 2 min at 200 x g and 1 min at 14,000 x g. Keep the flow-through. Eluted RNA is now ready for downstream analysis or for storage at -80°C.

Sensitivity:

The purified exosome RNA can be quantified and analyzed using a spectrophotometer, although the measured concentration values are likely to be towards the bottom limit of detection of the instrument. For better quantification, we recommend the concomitant use of electropherogram-based technologies or fluorometric technologies.