PRODUCT INFORMATION & MANUAL

0.4 micron Immunobeads for Overall Exosome Isolation (Mouse Biofluids)

NBP2-49831

For research use only.
Not for diagnostic or therapeutic procedures.

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I. Introduction:

Exosomes are small endosome derived lipid nanoparticles (50-120 nm) actively secreted by exocytosis by most living cells. Exosome release occurs either constitutively or upon induction, under both normal and pathological conditions, in a dynamic, regulated and functionally relevant manner. Both the amount and molecular composition of released exosomes depend on the state of a parent cell. Exosomes have been isolated from diverse cell lines (hematopoietic cells, tumor lines, primary cultures, and virus infected cells) as well as from biological fluids in particular blood (e.g. serum and plasma from cancer patients) and other body fluids (broncho alveolar lavage fluid, pleural effusions, synovial fluid, urine, amniotic fluid, semen, saliva etc). Exosomes have pleiotropic physiological and pathological functions and an emerging role in diverse pathological conditions such as cancer, infectious and neurodegenerative diseases.

Novus Immunobeads are useful for capturing and isolating overall or specific exosome sub-populations. Latex immunobeads are covalently coupled with antibodies against exosome surface antigens, allowing exosome capture from human biofluids (tested for plasma, serum and urine) and cell culture media without pre-purification steps (ultracentrifuge or other methods for exosome purification). Novus immunobeads are able to capture the overall exosome population (Immunobeads for Overall Exosome capture) or to enrich exosome subpopulation derived from a tumoral source (Tumor-derived exosome capture and enrichment). Immunobeads are supplied with an Exosome Elution Buffer, which allows detachment and elution of captured exosomes for downstream analyses, and with a Bead Regeneration Buffer to regenerate immunobeads for further usage. All latex Immunobeads are available in two sizes (0.4 and 1.0 micron of diameter) and are sold in packages of 3, 5, 10 and 20 reactions. The latex immunobeads are pre-coated with anti-mouse or anti-Rabbit antibodies. It uses an easy, fast and efficient protocol.

Beads are mixed together with the sample and incubated overnight (ON) for exosomes binding. After ON incubation beads can be easily recovered by centrifugation and washed with appropriate buffer. Bead pellet can be directly used for nucleic acid extraction or protein analysis. Alternatively, exosomes can be eluted off the beads with the Exosome Elution Buffer and used for functional studies.

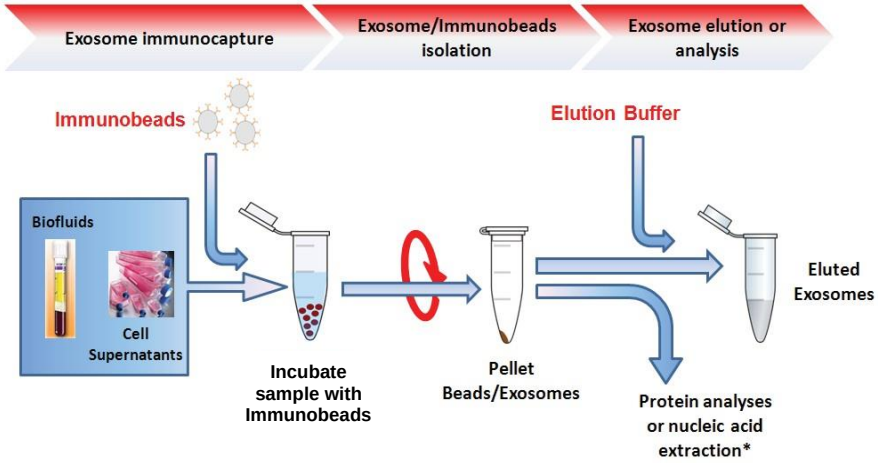

Figure 1. Immunobeads for Overall Exosome Isolation. Novus immunobeads are able to capture the overall exosome population (Immunobeads for Overall Exosome capture) or to enrich exosome subpopulation derived from a tumoral source (Tumor-derived exosome capture and enrichment).

II. Application:

- For overall exosome isolation from Mouse plasma or serum).
- Small sample volume of mouse biofluids.
- No ultracentrifugation or other methods for exosome purification required.
- Immunobeads are ready to use and stable for long term (up to 8 months).
- Immunobeads are suitable for nucleic acid extraction, protein profiling of exosome markers, exosome elution from immunobeads.
- A mouse monoclonal antibody has been used for coating

III. Sample Type:

- Mouse biofluids: Plasma, Serum.
- Recommended starting volume from 0.5 ml of sample upto 1 ml.
- Unfractionated plasma sample can be directly used for capture.

IV. Package Contents (for Overall Exosomes capture from mouse plasma and serum):

Components	NBP2-49831	
Pre-coupled Latex Immunobeads	100 µl (10 reactions)	200 µl (20 reactions)
Exosome Elution Buffer	1 bottle (250 µl)	1 bottle (450 µl)
Bead Regeneration Buffer	1 bottle (5 ml)	1 bottle (10 ml)

V. User Supplied Reagents and Equipment:

- Single-use and/or pipettes with disposable tips 2-100 µl.
- 1X PBS

VI. Shipment and Storage:

- Immunobeads are shipped and stored at 4°C for up to 8 months. DO NOT FREEZE!

VII. Reagent Preparation and Storage Conditions:

- Immunobeads (10 and 20 reaction packages) are supplied with Exosome Elution Buffer, for eluting intact exosomes from beads and with Bead Regeneration Buffer, for regenerating immunobeads that can be reused at least once more.

VIII. 0.4 micron Immunobeads for Overall Exosome Isolation (Mouse Biofluids) Protocol:

1. **Mouse Plasma and Serum sample preparation:** Prepare samples by 3 centrifugation steps to eliminate red blood cells and cellular debris. After each step, transfer the supernatant to a new tube and discard the pellet.

a) 10 min at 300g at 4°C (save the supernatant; discard pellet).
b) 20 min at 1200g at 4°C (save the supernatant; discard pellet).
c) 30 min at 10,000g at 4°C (save the supernatant; discard pellet). Plasma can be diluted 1/1 in 1X PBS.

2. **Exosome immunocapture:** Add 10 µl of pre-coupled beads to 0.5 ml up to 1 ml of biological sample (plasma, urine or cell culture supernatant previously precleared. Incubate overnight at 4°C in rotator. Remark: Incubation can be carried out also at room temperature for at least 4 hr in rotator. After exosome binding wash beads twice with 1 ml of PBS resuspending up and down. 10-15 times. In each step remove the supernatant by centrifugation at 5000g for 10 min. The prepared beads can be used for further captured exosome characterization including both protein and nucleic acid content analysis, or exosomes can be recovered and analyzed.
3. **Exosome elution from beads (Only for 0.4 micron bead size and for 10 and 20 reactions only).**

a) Add 10 µl of Exosome Elution Buffer, vortex for 30 sec. Incubate at RT for 5 min. Vortex again 30 secs and add 40 µl of 1X PBS.
b) Centrifuge 10 min at 5000g, transfer the supernatant in a clean tube (low binding) and store in ice.
c) Repeat the elution step as indicated in step 1. Centrifuge as indicated above.
d) Collect the two fractions of supernatant all together.

4. **Beads regeneration***

a) Add 500 µl of regeneration buffer, incubate for 5 min at RT.
b) Centrifuge 10 min at 5000g, discard the supernatant.
c) Wash beads with 1 ml of PBS, centrifuge as indicated above, discard the supernatant.
d) Resuspend beads in 10 µl of PBS.
* Beads can be reused for not more than twice.

e) **Sensitivity: Immunobeads are a useful tool for exosome protein profiling.** For Western Blotting analysis, we suggest to elute exosomes directly in Laemmli buffer and loaded on polyacrylamide gel as indicated in the example below (Figure 2). Exosome were detected with an anti-Alix antibody (human plasma and cell supernatant as described in Figure 2 and 3 respectively). **Latex Immunobeads are suitable for exosome RNA extraction and RNA marker profiling as shown in Figure 4**. Isolated total RNA is suitable for mRNA or small RNA analysis. Figure 4 shows the expression profile of TM9SF4 transcript in cancer cell derived exosomes. Exosomes can be successfully eluted from immunobeads which can be reused two more times. Exosomes immunocaptured from 1 ml of human plasma using latex beads were eluted with Exosome Elution Buffer and then quantified onto Novus immunoplate for overall exosome capture from plasma (Figure 5). Detection was performed using a proprietary anti-CD9-Biotin conjugated antibody. Eluted beads can be regenerated with Bead Regeneration Buffer and reused for capturing exosome two more times (Figure 6).

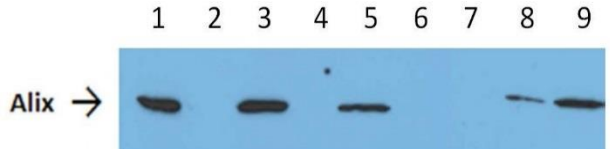

Figure 2. Alix expression by western blotting of human plasma exosomes captured on Novus Immunobeads from 0.5 ml of plasma in comparison with exosomes purified via ultracentrifuge. Lane 1, 3 and 5: Immunocapture with immunobeads from 1 ml, 0.5 ml and 100 µl of human plasma respectively. Lane 2, 4, 6: Ultracentrifuged exosomes from plasma after immunobeads capture. Lane 7: Immunocapture with immunobeads isotype coupled. Lane 8 and 9: Ultracentifuged exosome from 0.5 ml and 1 ml of human plasma.

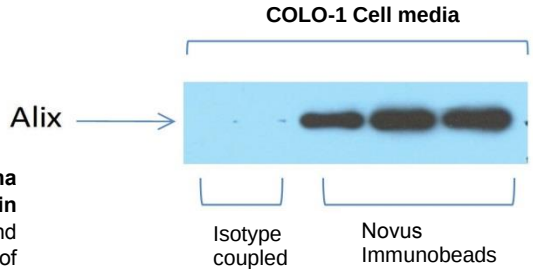

Figure 3. Alix expression by western blotting of exosomes captured on Novus immunobeads from COLO-1 cell supernatant vs isotype coupled beads.

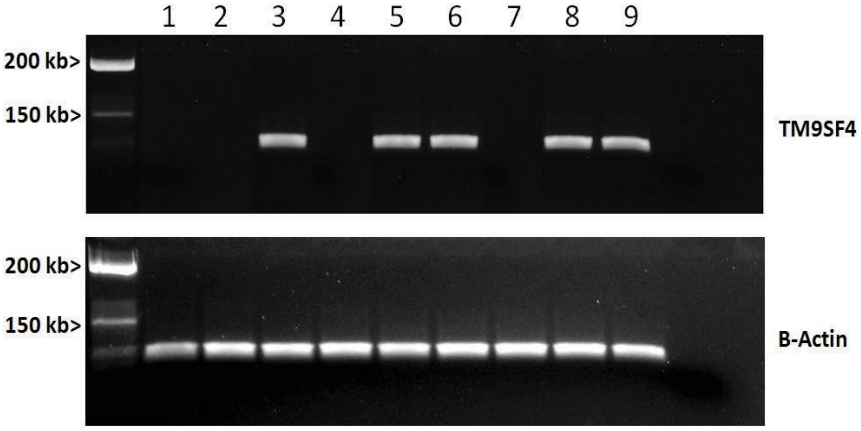

Figure 4. Profile of two mRNA marker in exosomes derived from 9 cancer cell lines immunocaptured using Novus immunobeads.

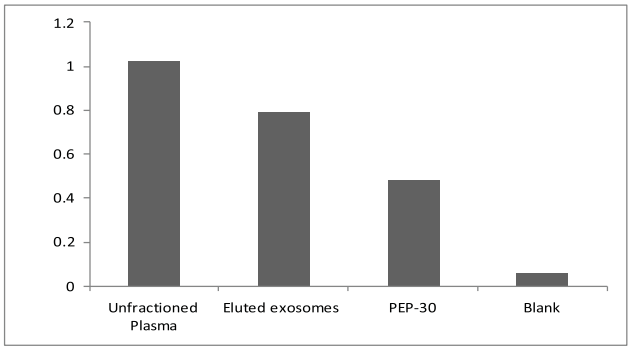

Figure 5. CD-9 expression in exosomes eluted from immunobeads.

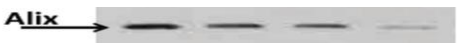

Figure 6. Western Blotting analysis of immunocaptured exosomes on beads. Lane 1: Exosomes immunocaptured with fresh beads. Lane 2: Exosomes immunocaptured with beads reused once. Lane 3: Exosomes immunocaptured with beads reused twice. Lane 4: Exosomes immunocaptured with beads reused the third time.

IX. General Troubleshooting Guide:

Problems	Cause/Solution
Low efficiency in immunobeads precipitation	Usage of low-binding tubes is recommended. Increase centrifugation time (15 min or 20 min at 5000g). Not increase centrifugation speed over 5000g.
Immunobeads damage	Conditions that would be damaging to the immunobeads include freezing (irreversible aggregation), high temperature (>95°C), exposure to organic solvents and high centrifugation speed (swelling, deformation, sticking of immunobeads).
Presence of aggregates and hard immunobeads resuspension:	Formation of small aggregates may happen collecting immunobeads after samples incubation. Small aggregates usually do not cause problems and can be resuspended by pipetting several time during washing steps. Incubation for 5 min at 37°C can help to disrupt small aggregates.
Irreversible aggregation in precipitation phase	Irreversible aggregation of immunobeads after incubation with biological samples may happen if samples are not well precleared or too concentrate. Preclear plasma samples following protocol indicated in appendix 1 before incubation with immunobeads. Dilute concentrated urine or cell culture supernatants samples with 1X PBS or a solution of NaCl (0.9 M).
Nonspecific absorption	Include or increase the percentage of Tween 20 in buffer for washing. Recommended concentrations: 0.05 % to 0.1%.
Exosomes markers detection in western blotting	Beads are coupled with mouse or rabbit antibody for exosomes immunocapture. The signal of heavy and light immunoglobulins chains can appear during the detection phase if a conventional secondary antibody HRP conjugated is used. The use of detection primary antibodies HRP or biotin tagged can eliminate this problem.