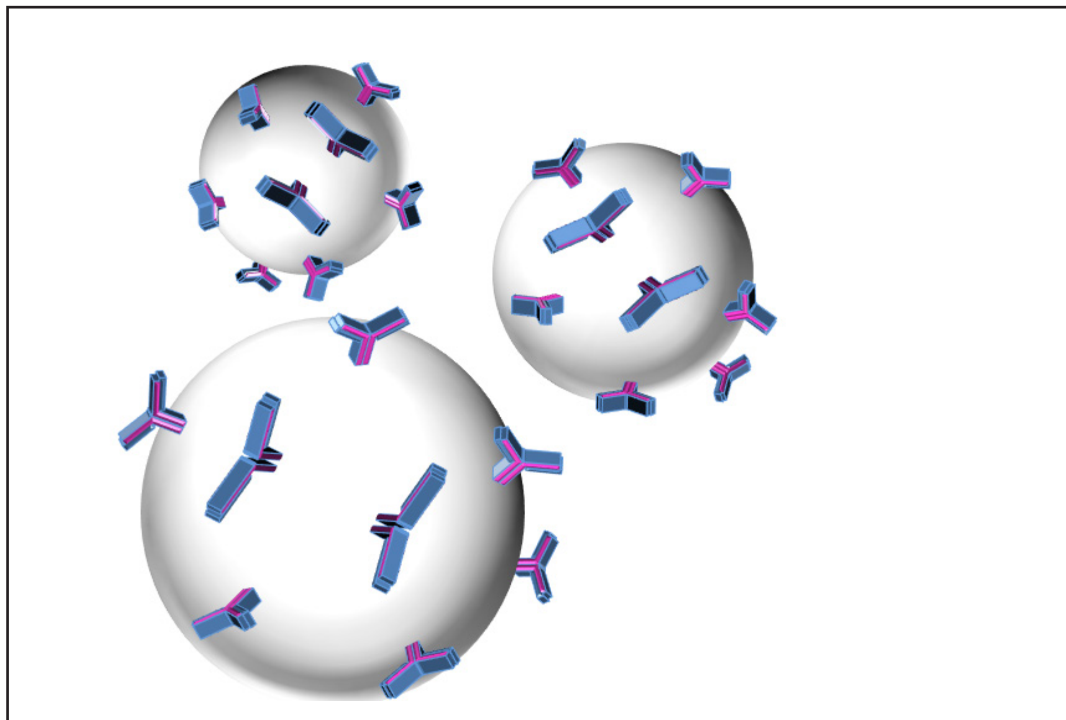
Datasheet

HansaBioMed



# Immunobeads for Exosome capture and isolation

This product is for research use only.
It is highly recommended to read this users guide in its entirety prior to using this product.
Do not use this kit or its components beyond the indicated expiration date.

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# PRODUCT DESCRIPTION

## Product overview

HBM provides several types of Immunobeads 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 method for exosome purification). HBM immunobeads are able to capture the overall exosome population (Immunobeads for Overall Exosome capture) or to enrich exosome subpopulation derived from tumoral source (Tumoral-derived exosome capture and enrichment). Immunobeads are supplied with an Exosome Elution Buffer, that allows detachment and elution of captured exosomes for downstream analyses, and with a Beads Regeneration Buffer to regenerate immunobeads for further usage.

**HBM Immunobeads are ready to use.**

## About Exosomes

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 amount and molecular composition of released exosomes depend on the state of a parent cell. Exosomes have pleiotropic physiological and pathological functions and an emerging role in diverse pathological conditions such as cancer, infectious and neurodegenerative diseases.

## Type of Immunobeads available:

Immunobeads for Overall Exosome capture from Human Biological fluids (plasma, serum and urine);
Immunobeads for Overall Exosome capture from cell culture supernatant;
Immunobeads for Tumor-derived Exosome capture from Human plasma and serum.

Available size 0.4, 1 microns.

| Content | Immunobeads 10 Reactions | Immunobeads 20 Reactions |
|---|---|---|
| Pre-coupled latex immunobeads | 1 vial (100 µl) | 1 vial (200 µl) |
| Elution buffer | 1 vial (250 µl) | 1 vial (450 µl) |
| Bead regeneration buffer | 1 bottle (5 ml) | 1 bottle (10 ml) |
| Expiration date | 8 months, store at 4°C | |
| * Elution buffer and Bead regeneration buffer are not provided with TEST immunobeads (3 and 5 reactions) | | |

## PROCEDURE

**Sample preparation:**

**Plasma and serum samples preparation**
Prepare samples by 3 centrifugation steps to eliminate red blood cells and cellular debris:

- 10’ at 300 g
- 20’ at 1 200 g
- 30’ at 10 000 g

**Urine samples preparation**
Preclear urine samples by centrifugation at 16 000g for 20’ at RT

- Filter by using 0.45 um filter
- Concentrate urine samples by spin concentrator 10 X (from 10 ml to 1 ml) for protein and for nucleic acid analyses*.

**Cell culture mediaum samples preparation**
Prepare cell supernatants by 3 centrifugation steps:

- 10’ at 300 g
- 20’ at 1 600 g
- 30’ at 10 000 g

Concentrate cell supernatant 10 X (from 10 ml to 1 ml) in spin concentrator*

**The quantity of exosomes could vary between samples. Concentration factors are given for information purposes only, a larger starting amount of sample should be used if the signal is weak.*

**Exosomes 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 in according with indications in appendix 1. Incubate overnight at 4°C in rotator.
- **Remark:** Incubation can be carried out also at room temperature for at least 4 hours 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 5 000 g for 10’.
- 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.

**Exosome elution from beads (Only for 0.4 and 1 micron bead size, 10 and 20 reactions)**

- Add 10 µl of Exosome Elution Buffer, vortex for 30’’, incubate at RT for 5’. Vortex again 30’’ and add 40 µl of PBS 1X.
- Centrifuge 10’ at 5 000 g, transfer the supernatant in a clean tube (low binding) and store in ice.

- Repeat the elution step as indicated in step 1.
- Centrifuge as indicated above.
- Collect the two fractions of supernatant all together.

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**Beads regeneration***

- Add 500 μl of regeneration buffer, incubate for 5' at RT.
- Centrifuge 10' at 5 000 g, discard the supernatant.
- Wash beads with 1 ml of PBS, centrifuge as indicated above, discard the supernatant.
- Resuspend beads in 10 μl of PBS.

* Beads can be reused for not more than twice.

## PERFORMANCE

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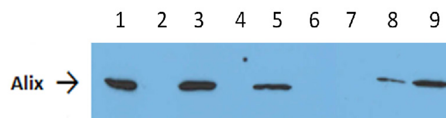
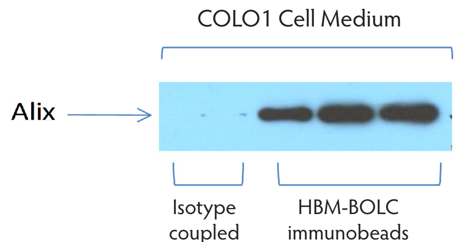
**Immunobeads are an useful tool for exosome protein profiling**

For **Western Blotting** analysis we sugest to elute exosomes directly in Laemmli buffer, as indicated in the example below. Following incubation, beads were recovered by centrifugation, resuspended in Laemmli buffer, and loaded on polyacrylamide gel for western blotting analysis. Exosome were detected with an anti-Alix antibody (human plasma and cell supernatant as described in fig 1 and 2 respectively).




1, 3 and 5: Immunocapture with immunobeads from 1 ml, 0.5 ml and 100 μl of human plasma respectively.
2, 4, 6: Ultracentrifuged exosomes from plasma after immunobeads capture.
7 Immunocapture with immunobeads isotype coupled.
8, 9: Ultracentifuged exosome from 0.5 ml and 1 ml of human plasma.

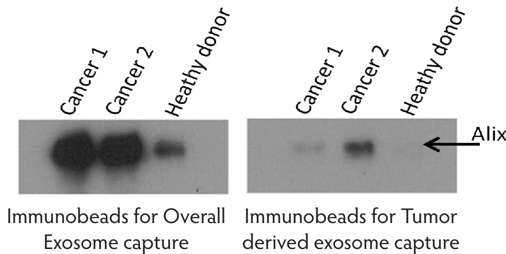
1. Alix expression by western blotting of human plasma exosomes captured on HBM-Immunobeads from 0.5 ml of plasma in comparison with exosomes purified via ultracentrifuge. 1, 3 and 5: Immunocapture with immunobeads from 1 ml, 0.5 ml and 100 μl of human plasma respectively. 2, 4, 6: Ultracentrifuged exosomes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




2. Alix expression by western blotting of exosomes captured on HBM-BOLC immunobeads from COLO1 cell supernatant vs isotype coupled beads.

**Immunobeads enrich for Tumor-derived exosome subpopulation in cancer patients**

Immunobeads for tumor-derived exosome capture can be used for enriching exosome subpopulations derived from tumoral sources thus providing a novel platform for cancer biomarker research.




3. Anti-Alix WB analysis on exosomes immunocaptured with beads for overall (HBM-BOLF) and for tumor-derived exosomes (HBM-BTLF). WB shows the capture of exosomes only for cancer patients when beads for tumor-derived exosomes are used.

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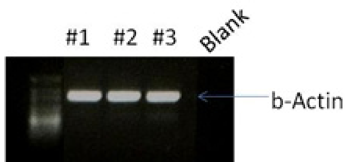
**Immunobeads can be used for exosome RNA extraction**

It is possible to extract RNA from exosomes isolated on immunobeads, lysing exosomes with a phenol based reagent (TRIzol, QIAzol or similar reagents). (See also RNA extraction Kits in HBM catalog).

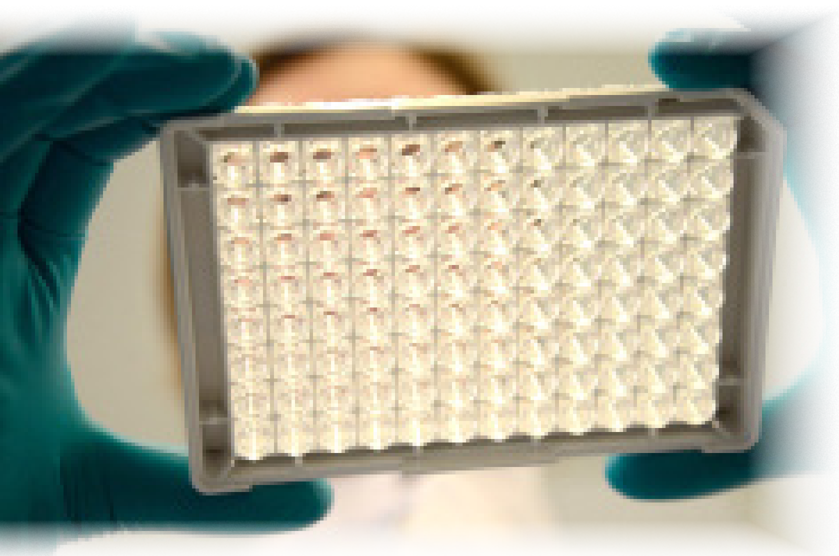



4. RT-PCR analysis of total exosomal RNA derived from exosomes immunocaptured by 0,5 ml of three different human plasma samples. Following immunocapture on HBM-immunobeads total RNA was extracted from vesicles using RNA Basic Kit (HBM), then retrotranscribed using RT-Script III (Invitrogen). B-Actin fragments were amplifiled with Emerald master-mix (Takara).

## TROUBLESHOOTING

**Contact us at: info@hansabiomed.eu**

| Problem/Cause | Suggested Solution |
|---|---|
| **Low efficiency in immunobeads precipitation** | Usage of low-binding tubes is recommended. Increase centrifugation time (15' or 20' at 5 000 g). Not increase centrifugation speed over 5 000 g. |
| **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 5' 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 PBS 1x 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 eliminate this problem. |



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