ab288113 – 1.0 micron Immunobeads

For the capture and isolation of overall or specific exosome sub-populations plasma, urine, serum and other biofluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
https://www.abcam.com/ab288113

Storage and Stability

The kit and contents can be stored at 4°C for up to 8 months. DO NOT FREEZE.

Materials Supplied

Item	Quantity	Storage Condition
Pre-coupled Latex Immunobeads	100 µl (10 reactions)	4°C
Exosome Elution Buffer	1 bottle (650 µl)	4°C
Bead Regeneration Buffer	1 bottle (10 ml)	4°C

Materials Required, Not Supplied

The following materials or reagents are not included in the kit, but will be required to successfully utilize this assay:

- 1X PBS

Reagent Preparation

- Immunobeads 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.

Sample Preparation

Human 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).
Δ Note: *Plasma can be diluted 1/1 in 1X PBS.*

Human Urine sample preparation: Preclear urine samples by centrifugation at 16,000g for 20 min at RT:

a) Filter by using 0.45 µm filter.
b) Concentrate urine samples by spin concentrator for 15-20 times for proteomic and for nucleic acid studies.
Δ Note: *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.*

Assay Protocol

Exosome immunocapture:

1. 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.
Δ Note: 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 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.

Exosome elution from beads (Only for 1.0 micron bead size and for 10 and 20 reactions only):

1. Add 10 µl of Exosome Elution Buffer, vortex for 30 secs. Incubate at RT for 5 min. Vortex again 30 secs and add 40 µl of 1X PBS.
2. Centrifuge 10 min at 5000g, transfer the supernatant in a clean tube (low binding) and store on ice.
3. Repeat the elution step as indicated in *Human Plasma and Serum sample preparation* above. Centrifuge as indicated above.
4. Collect the two fractions of supernatant all together.

Beads regeneration:

1. Add 500 µl of regeneration buffer, incubate for 5 min at RT.
2. Centrifuge 10 min at 5000g, discard the supernatant.
3. Wash beads with 1 ml of PBS, centrifuge as indicated above, discard the supernatant.
4. Resuspend beads in 10 µl of PBS.
Δ Note: Beads cannot be reused more than two times.

Sensitivity

- Immunobeads are a useful tool for exosome protein profiling. For Western Blotting analysis, we suggest eluting exosomes directly in Laemmli buffer and loading onto a polyacrylamide gel. Exosomes are detected using an anti-Alix antibody.
- Latex Immunobeads are suitable for exosome RNA extraction and RNA marker profiling.
- Isolated total RNA is suitable for mRNA or small RNA analysis.
- Exosomes immunocaptured from 1 ml of human plasma using latex beads are eluted with Exosome Elution Buffer and can then be quantified for overall exosome capture from plasma.
- Detection can be carried out using a proprietary antiCD9-Biotin conjugated antibody. Eluted beads can be regenerated with Bead Regeneration Buffer and reused for capturing exosome two more times.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)