ab285284 – Overall Exosome Capture and Quantification ELISA Assay Kit

For the comprehensive profiling of exosomes.
For research use only – not intended for diagnostic use

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285284

Storage and Stability

On receipt entire assay kit should be stored at 4°C, protected from light. DO NOT FREEZE.

Materials Supplied

Item	Quantity	Storage Condition
Sample buffer	2 x 10 mL	4°C
25X Washing buffer	25 mL	4°C
Primary antibody	20 µL	4°C
HRP-conjugate	1 Vial	4°C
Substrate chromogenic solutions	10 mL	4°C
Stop solution	10 mL	4°C
Exosome Standards	2 Vials	4°C
Immunoplate (Transparent)	1 Unit	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
- Polypropylene tubes
- Pipettes 1 mL and 5 mL for reagent preparation
- Deionized water
- PBS
- Plate shaker
- Humidified chamber or incubator at 37°C
- Disposable pipetting reservoirs
- Microplate reader
- ELISA sealing film or parafilm

Reagent Preparation

- Dilute the 25X Washing Buffer to 1X with deionized water. If crystals are observed, dissolve them by warming up the vial at 37°C the solution before preparing a dilution.
- Reconstitute lyophilized exosome standard by adding 100 µl of deionized water, pipette the solution up and down 10-15 times, avoiding bubbles. Vortex the reconstituted standard for 60 sec. Briefly centrifuge the tubes containing standards to ensure that the solution is collected at the bottom of the tube. Pipette the solution up and down 10 times, again avoiding any bubbles.
- If purified exosome samples are analyzed, use 1X PBS to adjust the volume and concentration of samples (overall volume/well is 100 µl).
- In general, unfractionated samples are analyzed without dilutions (100 µl/well). If OD values observed, are beyond the range of standard curve. Dilute the samples using 1X PBS.
- Detection antibody should be diluted to 500-fold in sample buffer.
- HRP-conjugated secondary antibodies should be diluted to 2000-fold in sample buffer.
- Substrate solution for colorimetric readings and stop solution are ready to use.
- The plate is packed in an opaque aluminum pouch which complies with the food and pharmaceutical regulation. The pouch is easy to open and is re-sealable by zip closure.
- ELISA strips: Unused strips should be placed back in the foil pouch with the included desiccant pack, resealed and stored at 4°C for up to one month.
- Exosome standards: The remaining reconstituted standard stock solution should be aliquoted into polypropylene vials (preferably low binding) and stored at -20°C for up to one month or at -80°C for up to six months. Strictly avoid repeated freeze-and-thaw cycles.
- .After opening, use within one month.

Assay Protocol

Human Plasma 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 supernatant; discard pellet).
b) 20 min at 1200g at 4°C (save supernatant; discard pellet).
c) 30 min at 10,000g at 4°C (save supernatant; discard pellet). Human plasma can be diluted 1/1 using 1X PBS prior to loading onto ELISA plates.

Reconstituted Exosome Standard for calibration curve:

Bring all the reagents to room temperature and briefly vortex the tubes before use. The positive control is represented by the highest concentration of exosome standards. The negative control is represented by sample buffer or 1X PBS for analysis of purified exosomes and sample matrix (e.g., exosome depleted cell culture supernatant or plasma) for unfractionated samples.

a) Reconstitute lyophilized exosome standard by adding 100 µl of deionized water in each vial.
b) Pipette the solution up and down 10-15 times, avoiding bubbles.
c) Vortex the reconstituted standard for 60 sec. Briefly centrifuge the tubes to spin down the contents and ensure that the solution is collected at the bottom of the tube.
d) Pipette the solution up and down 10 times, again avoiding any bubbles.
e) Briefly centrifuge again. Add 100 µl of 1X PBS to reach a final volume of 200 µl per vial.

Standard Curve Preparation:

Standard dilutions are prepared directly in the strips. Please use the exosome stock solution prepared as indicated above to perform six two-fold serial dilutions using 1X PBS. The standard concentrations in the wells will be represented as 50 µg, 25 µg, 12.5 µg, 6.25 µg, 3.125 µg, 1.5625 µg and 0.78125 µg respectively.

1. Add 200 µl of the reconstituted exosome solution to wells A1 and A2 (2 wells only).
2. Add 100 µl of 1X PBS to wells B1 to H2 (14 wells).
3. Serial dilution (stop at G1 and G2).
4. Transfer 100 µl of A1 into B1 and mix.
5. Transfer 100 µl of B1 into C1 and mix.
6. Transfer 100 µl of C1 into D1 and mix.
7. Transfer 100 µl of D1 into E1 and mix.

8. Transfer 100 µl of E1 into F1 and mix.
9. Transfer 100 µl of F1 into G1 and mix.
10. Discard 100 µl from G1 to result in a final volume of 100 µl.

    Δ Note: Leave H1 (and H2) as 1x PBS for negative controls.

11. Repeat serial dilution for A2 to G2.

Exosome binding:

Add 100 µl of prepared samples to wells A3 to H12 (add 1X PBS if the volume is less than 100 µl). Seal the plate with a parafilm and incubate at room temperature while shaking for 30 min (2-3 rotations per sec). Transfer the plate to 4°C and incubate overnight (12 hr-20 hr) (for human plasma samples).

Plate Washing step:

Add 200 µl/well of Washing Buffer and discard the plate contents by pouring out. Wash three times with 300 µl/well of Washing Buffer. After each addition, pour off wash. All subsequent washings should be performed in the same manner.

Primary antibody binding:

Wash the plate as indicated above. Then add 100 µl of mouse anti-human exosome Detection Antibody solution (diluted in Sample Buffer at 1:500 dilution) to each well. Seal the plate with parafilm and incubate at room temperature while shaking for 15 min (2-3 rotations per sec). Then incubate for 2 hr at 4°C (for human plasma samples).

Wash the plate as described above. Add 100 µl of rabbit anti-mouse IgG HRP-conjugated secondary antibody solution to each well (diluted to 1:2000 dilutions in 1X Sample Buffer). Seal the plate with parafilm and incubate at room temperature while shaking for 15 min (2-3 rotations per sec). Then incubate for 1 hr at 4°C (for human plasma samples). Wash the plate as indicated above.

For Colorimetric detection:

Add 100 µl of Substrate Chromogenic Solution to each well and incubate at room temperature in the dark for 5-10 min. Be careful not to immerse metallic components of a pipette into the substrate solution. Also avoid making bubbles and, if formed, remove them gently with a pipette tip. Do not seal the plate and monitor till a blue color is visible. The intensity of the color is proportional to the exosome concentration only within a certain dynamic range. Many plate readers do not deliver accurate results when the OD is above 3. Stop the reaction by adding 100 µl of Stopping Solution to each well. The color will change from blue to yellow.

Read the absorbance at 450 nm within 10 min. If possible, the absorbance should also be read at 570 nm and the measurement should be subtracted from the measurement at absorbance 450 nm.

Calculation:

Exosome standards are provided as assay calibrators and also as the positive control. It is important to note that the origin of purified standard exosomes may change the proportion of common exosomal proteins such as CD9. The amount of proteins on their membrane might differ slightly from the amount on the sample exosomes. The standard curve is used to determine the amount of exosomes in an unknown sample. The curve is obtained by plotting the average readings for different standard concentrations against the corresponding amounts of exosomes.

Calculate the mean absorbance for each set of duplicate standards, controls, and samples. The values of the negative controls (blanks) must be subtracted from all OD values before the results can be interpreted. The regression curve coefficient should be above 0.95. The estimated sample concentration is reliable if within the linear range of the curve, otherwise the samples must be diluted and the test repeated. For diluted samples, multiply the concentrations with the appropriate dilution factors.

Sensitivity:

The data reported demonstrates that the sensitivity is higher than that of Western blotting.

Technical Support

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