ab287851 – ExoQuant Overall Exosome Capture and Quantification Assay Kit (Colorimetric)

For quantitative and qualitative analysis of exosomes from cell culture supernatant.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287851

Storage and Stability

On receipt entire assay kit should be stored at 4°C, and used within 24 months. Do not Freeze.

Materials Supplied

Item	Quantity	Storage Condition
Sample buffer (1X)	2 x 10 ml	4°C
Washing buffer (25X)	25 ml	4°C
Primary antibody	20 µl	4°C
HRP conjugated antibody	5 µl	4°C
Substrate chromogenic solutions	10 ml	4°C
Stop solution	10 ml	4°C
Exosome standards	2 x 100 µg	4°C
Immunoplate (Transparent)	1 plate	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

- Before using the kit, bring all the reagents to room temperature (15-30 mins)

Washing Buffer: Dilute the 25X Washing Buffer to 1X with deionized water. If crystals are observed, dissolve them by warming up the vial at 37°C before preparing a dilution.

Detection Antibody: Dilute to 500-fold in sample buffer.

HRP-Conjugated Antibody: Dilute to 5000-fold in sample buffer.

Substrate Solution: Ready to use.

Stop Solution: Ready to use.

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.

ExoQuant Assay Protocol

Cell Culture Medium Sample Preparation: In general, unfractionated samples are analyzed without dilutions (100 µl/well). If the OD values observed are beyond the range of standard curve, dilute the samples using 1X PBS.
Prepare samples by 3 centrifugation steps at 4°C. After each step, transfer the supernatant to a new tube and discard the pellet.

a. 10 min at 300g
b. 20 min at 1200g
c. 30 min at 10,000g

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.*

Reconstitution of Exosome Standard: Reconstitute lyophilized standard by adding 100 µl of deionized water to each of the tubes and pipetting 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 bubbles. Briefly centrifuge again. Add 100 µl of 1X PBS to reach a final volume of 200 µl per vial.

Δ Note: *The remaining reconstituted standard stock solution after use 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.*

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.

a. Add 200 µl of reconstituted exosome solution to wells A1 and A2 (2 wells only).
b. Add 100 µl of 1X PBS to wells B1 to H2 (14 wells).
c. Transfer 100 µl of A1 into B1 and mix.
d. Transfer 100 µl of B1 into C1 and mix.
e. Transfer 100 µl of C1 into D1 and mix.
f. Transfer 100 µl of D1 into E1 and mix.
g. Transfer 100 µl of E1 into F1 and mix.
h. Transfer 100 µl of F1 into G1 and mix.
i. 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.

Repeat serial dilution for A2 to G2.

Exosome Binding: Add 100 µl of prepared samples to wells A3 to H12, adding 1X PBS if required to make the volume up to 100 µl. Seal the plate with parafilm and incubate at room temperature while shaking for 30 min (2-3 rotations per sec). Transfer the plate to

+37°C and incubate overnight (12 hr-20 hr) in a humid chamber (for cell culture medium samples).

Washing Procedure: Wash the plate by adding 200 µl of Washing Buffer to each well, then discard the plate contents by pouring it out into a sink or waste disposal. Repeat this operation three times with 300 µl/well of Washing Buffer. Do not touch the wells at any time. All subsequent washings should be performed in the same manner.

Primary Antibody Binding: Add 100 µl of the diluted mouse anti-human exosome Detection Antibody solution 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 hours at 37°C (for cell culture medium samples). After the incubation, repeat the washing procedure above.

HRP-Conjugated Antibody Binding: Add 100 µl of the diluted Streptavidin-HRP antibody solution 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 1 hr at 37°C (for cell culture medium samples). After the incubation, repeat the washing procedure above.

Colorimetric Detection: Add 100 µl of Substrate Chromogenic Solution to each well and incubate at room temperature, in the dark, for 5-10 mins. Be careful not to immerse metallic components of a pipette into the substrate solution. Avoid making bubbles and, if formed, remove them gently with a pipette tip. Do not seal the plate. Continue to monitor the plate until a blue color is visible. The intensity of 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 minutes. If possible, the absorbance should also be read at 570 nm and the measurement should be subtracted from the measurement of absorbance at 450 nm.

Calculation:

- Exosome standards are provided as assay calibrators and 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 amounts of proteins on their membrane might differ slightly from the amounts on the sample exosomes. The standard curve is used to determine the amount of exosomes in an unknown sample; this 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.

Technical Support

For all technical or commercial enquiries please go to:
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