CREATIVE BIOMART®
Assay Kit

Overall Exosome Capture and Quantification Assay Kit (Cell media, Colorimetric)

Product Information

Common Name

Exosome

Cat.No. Kit-1108

Product Overview

Kit-1108 is a patented double sandwich enzyme-linked immunoassay for quantitative and qualitative analysis of exosomes. It is a successful platform for exosomes quantification and characterization from small amount of human biological fluids (plasma, serum, urine, saliva) or cell media. It may be exploited to identify exosomes released by cancer cells in the plasma and urine of tumor patients in various disease conditions. Kit-1108 consists of ELISA plates pre-coated with proprietary pan-exosome antibodies enabling specific capture of exosomes from different biological samples, including cell culture supernatants and human biological fluids. Quantification and characterization of exosomal proteins is subsequently performed using appropriate detection antibodies against exosome associated antigens that can be for either generic or cell/tissue-specific exosomes. The kit contains Kit contains Lyophilized Exosome Standards for assay calibration. Lyophilized Exosome Standards, characterized for protein content and particle number allow the quantification of unknown sample by a standard calibration curve. Transparent and white plates are available depending on the downstream detection approach (colorimetric and luminometric respectively).

Description

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

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physiological and pathological functions and an emerging role in diverse pathological conditions such as cancer, infectious and neurodegenerative diseases.

Applications

• Kits ensure dose-dependent exosome detection in a variety of samples. No initial exosome purification required. The quantification can be both relative and absolute as the assay calibration enables the calculation of the approximate exosome quantity for each sample.
• Exosome comprehensive profiling.
• Pre-clinical researchon non-invasive biomarkers for detection and monitoring of anumber of pathologicalconditions (inflammation, cancer, neurodegeneration, etc). User friendly and suitable for multiple marker analyses.
• Up to 100 µl of biological sample can be loaded per well. Concentrated cell culture supernatant samples are recommended prior capture according to our suggested protocol.

Storage

All the reagents are shipped and stored at 4°C for up to 24 months, if unopened. Briefly centrifuge small vials prior to opening. DO NOT FREEZE!

Size

100 assays

Kit Components

Sample Buffer (1X): Buffer for antibody dilution and incubation, 2 bottles (2 x 10 ml)
Washing Buffer (25X): Buffer for washing plate, 1 bottle (25 ml)
Primary Antibody: a-CD9 mouse antibody, 1 vial (20 µl)
HRP Conjugated: Streptavidin HRP-conjugated secondary antibody, 1 vial (5 µl)
Substrate Chromogenic Solutions: Substrate for chromogenic detection, 1 bottle (10 ml)
Stop Solution: Stop solution, 1 bottle (10 ml)
Exosome Standards: Lyophilized exosomes from COLO-1 cell culture supernatant, 2 vials (2 x 100 µg)
Immunoplate (Transparent): Immunoplate standard multiwell plates 96-well format where assays can be conducted as singletons and/or multiple wells, 1 plate

Materials Required but Not Supplied

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- 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

Compatible Sample Types

Cell culture supernatant

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 seconds. 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.
- 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.
- Streptavidin-HRP-conjugated secondary antibodies should be diluted to 5000-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 food and pharmaceutical regulation. The pouch is easy to open and is re-sealable by zip closure.

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• 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.
• Store opened and diluted reagents at +4°C up to 24 months if unopened. After opening, use within one month.

Assay Protocol

1. Cell-culture medium Sample Preparation: Prepare cell supernatants by 3 centrifugation steps:
a) 10 min at 300 g at 4°C (save supernatant; discard pellet).
b) 20 min at 1600 g at 4°C (save supernatant; discard pellet).
c) 30 min at 10,000 g at 4°C (save supernatant; discard pellet).
Concentrate cell supernatant 10-20 fold in spin concentrator*. *The quantity of exosomes could vary between samples. A larger starting amount of sample should be used if the signal is weak.
2. Reconstituted Exosome Standard for calibration curve: Bring all the reagents to room temperature 15-30 min 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 drops and ensure that the solution is collected at the bottom of the tube.
d) Pipette the solution up and down 10 times, again avoiding bubbles.
e) Briefly centrifuge again.
f) Add 100 µl of 1X PBS to reach a final volume of 200 µl per vial.
3. 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 (see figure below). 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.

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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) Serial dilution (stop at G1 and G2).
d) Transfer 100 µl of A1 into B1 and mix.
e) Transfer 100 µl of B1 into C1 and mix.
f) Transfer 100 µl of C1 into D1 and mix.
g) Transfer 100 µl of D1 into E1 and mix.
h) Transfer 100 µl of E1 into F1 and mix.
i) Transfer 100 µl of F1 into G1 and mix.
j) Discard 100 µl from G1 to result in a final volume of 100 µl.
k) NOTE: Leave H1 (and H2) as 1x PBS for negative controls.
l) Repeat serial dilution for A2 to G2

4. 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 +37°C and incubate overnight (12 hr-20 hr) in humid chamber (for cell culture medium samples).
5. Wash the plates as shown below. Add 200 µl/well of Washing Buffer and discard the plate contents by pouring out. No Touch. Wash three times with 300 µl/well of Washing Buffer. After each addition, pour off wash. No Touch. All subsequent washings should be performed in the same manner.
6. 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 37°C in humid chamber (for cell culture medium samples).
7. Wash the plate as indicated above. Add 100 µl of Streptavidin-HRP antibody solution to each well (diluted to 1:5000 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 37°C in humid chamber (for cell culture medium samples). Wash the plate as indicated above.
8. For Colorimetric detection: Add 100 µl of Substrate Chromogenic Solution to each well and

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incubate at room temperature in the dark for 5-10 min. Be careful not to immerse metallic components of a pipette into 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. 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 at absorbance 450 nm.
9. 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.
10. Reproducibility: Intra Assay (within run) CV (%) < 10 Inter Assay (interplate run) CV (%) < 13. Coefficient of variation (CV) is expressed as a percentage of variance to the mean calculated for intra assay reproducibility evaluation by assessing at least 4 replicates of three different standard preparations containing different exosome concentrations. Subsequently, assay to-assay reproducibility was assessed by comparing mean absorbance for the same samples on independent plates tested by different operators.

Sensitivity

The sensitivity of the kit was compared to Western blot. The data reported demonstrate that the sensitivity of the kit is higher than that of Western blotting.