ab326322 – Exosome ELISA kit

For research use only - not intended for diagnostic use.

Product Description:

Exosome ELISA kit is an assay based on the use of antigens or antibodies labelled with an enzyme, so that the resulting conjugates have both immunological and enzymatic activity. As one of the components (capture antibody) is coating a support (immunosorbent) the antigen antibody reaction will remain immobilized and therefore, is easily revealed by the addition of a specific substrate.
After incubation with the sample and subsequent washing, an antigen-specific HRP-conjugated antibody is added. After an intermediate wash to remove any excess antibody, a colorimetric reaction mediated by the HRP enzyme is observed with the naked eye when the substrate solution (TMB) is added. This reaction can be quantified using a spectrophotometer or colorimeter. The following protocol describes how a direct ELISA is performed.

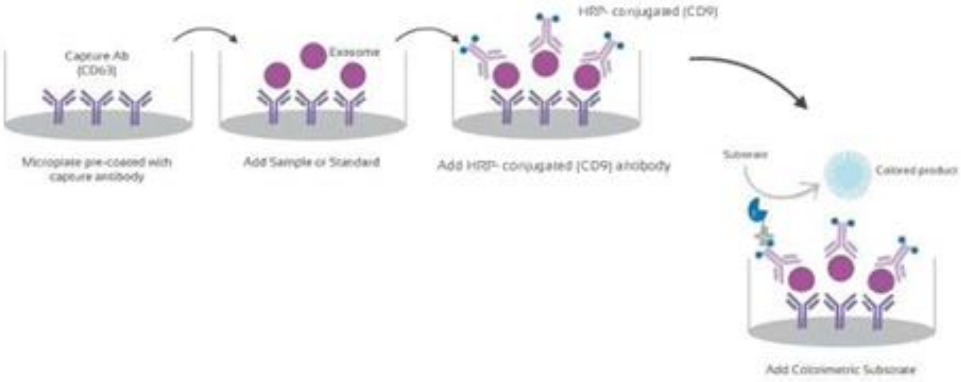

Figure 1: Indirect ELISA graphical representation

Storage and Stability:

The entire kit may be stored in the dark at 4ºC. The kit is stable until the expiry date stated on the box label if kept at 4ºC. Do not use after the date indicated. For prepared reagent storage, see table below.

Materials Supplied

Item	Quantity	Storage Condition
Immunoplate 96-well (coated with CD63)	1 Unit	4ºC
Standard for assay calibration: Lyophilized Human Exosome Standards	100 µg	4ºC
Washing buffer 25X	50 mL	4ºC
Superparamagnetic Capture Beads	25 mL	4ºC
Buffer diluent 1X	25 mL	4ºC
Primary Antibody	120 µL	4ºC
Biotin conjugated HRP-Conjugated antibody	120 µL	4ºC
TMB	12 mL	4ºC
H2SO4 0.5M: Stop solution	12 mL	4ºC

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Calibrated spectrophotometer for reading ELISA plates at 450nm and 630nm.
- Precision pipettes to deliver 1- 1000 µl volumes.
- Automatic plate washer: recommended. Plate washing can also be performed manually.
- Incubator: for incubation of the microplate at 37ºC.
- Distilled or deionized water.
- Timer
- Disposable gloves
- Waste container for biological substances
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

Standard Preparation

The kit contains a vial of lyophilized exosomes (100 µg) with which you can build your calibration curve.
Lyophilized exosomes can be stored at 4ºC for up to 2 years without functional compromise. It is recommended to store small, single –use aliquots of reconstituted exosomes, at – 20ºC for up to one month or at – 80ºC for longer periods, preferably in locations in frost-free freezers, without appreciable temperature fluctuation. This will minimize protein denaturation that can occur after multiple freeze/thaw cycles.

Reconstituted exosomes, stored properly, are functionally guaranteed for up to six months from date of reconstitution. Any unfrozen and/or unused exosome standard can be stored at 4ºC for short term use (<1 week), and should not be re-frozen.

To carry out the dilutions for the construction of the standard curve follow the protocol below:

1. Resuspend the standard vial in 100 µl of deionized water and let it reconstitute for at least 15 minutes. It is recommended to do each of the dilutions in duplicate to minimize the error. We will make the dilutions in Eppendorf and then, we will transfer them to the ELISA plate wells µl/well).
2. To perform the dilution in duplicate, we will add 60 µl of the lyophilized resuspended (100 exosomes in Eppendorf nº1. We will complete with 340 µl of dilution buffer and homogenize the sample. The rest of Eppendorf must contain 200 µl of dilution buffer.
3. We need to mix 200 µl from Eppendorf nº1 (1/1 dilution) to Eppendorf nº2 (1/2 dilution). Repeat this process until the 1/1024 dilution is completed (figure 2). Last well should contain only diluent buffer. This is the point that we will use as blank.

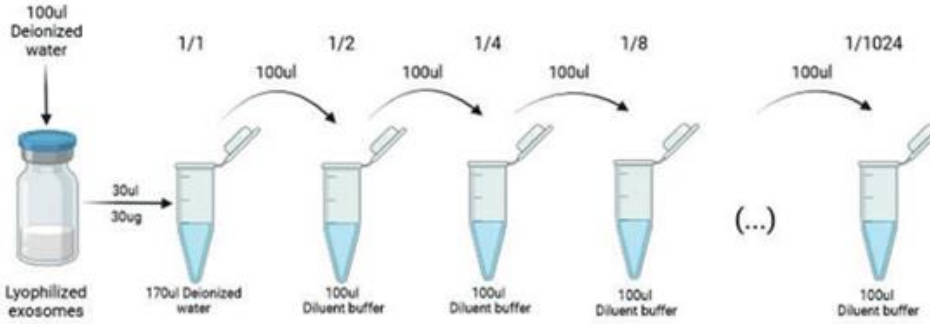

Figure 2: Graphical representation of the serial dilution of the Standard included in this kit.

Note: We recommend between 7 and 11 points to have a better model fit. The curve should always have a blank value, in order to avoid future problems with sample interpolation.

Sample Preparation

Depending on the reference to be used, we recommend processing the sample as follows:

A. <u>Sample pretreatment for direct exosome detection on cell culture supernatant.</u>
The sample pretreatment for direct exosome detection from cell culture supernatant is not recommended for detection of exosomes from any other body fluids. To ensure that detected exosomes originate from your cells of interest, culture the cells with exosome depleted fetal bovine serum (FBS), because normal FBS contains extremely high levels of exosomes that will contaminate the cells derived exosomes.

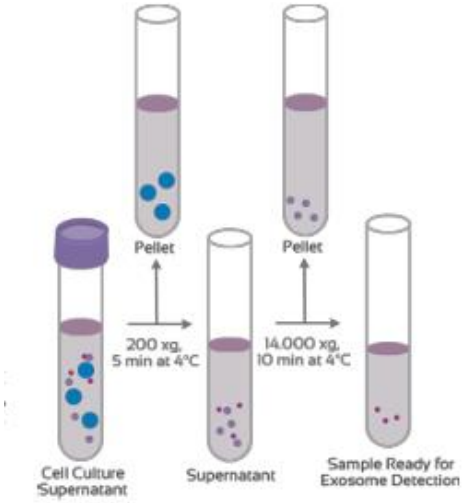

Figure 3: Cell Culture Supernatant pretreatment workflow for direct exosome detection

B. <u>Sample pretreatment for direct exosome detection on Serum/Plasma/Urine.</u> The sample pretreatment for direct exosome detection from plasma or serum is not recommended for detection of exosomes from any other body fluids or cell culture media.

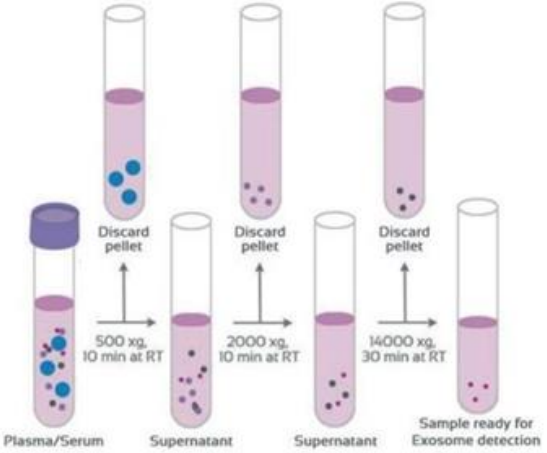

Figure 4: Serum Supernatant pretreatment workflow for direct exosome detection

If the OD of the sample is greater than the highest concentration point, we recommend to perform a dilution until the values fit the model we are following.

Procedure

1. Sample addition – Add 100 µl of the prepared sample (1:100 dilution with sample diluent) into the individual wells of the microplate. It is recommended to use two wells per sample. Incubate for 2 hours at 37° C (or overnight at 4°C). When the process is manual, cover the microplate with one of the protective sheets provided.
2. Washing - If necessary, remove the protective foil. Empty the wells and then wash 4 times using 3 00 µl of 1X wash buffer for each wash. Leave wash buff er in each we ll for 30-60 seconds for each wash cycle. After washing, completely remove all liquid from the microplate by tapping it on absorbent paper with the openings facing down to remove all residual wash buffer.
3. Antibodies binding - Add 100ul/well of the antibody dilution (1:100 in sample diluent). Seal the plate with parafilm and incubate an hour at +37°C.
4. Washing - If necessary, remove the protective foil. Empty the wells and wash as described previously (step 2).
5. Substrate Incubation - Add 100 µl of the Chromogen Substrate Solution (TMB) to each well of the microplate. Incubate for 10 minutes at room temperature (18 ° C and 24 ° C) and protected from light.
6. Stopping - Add 100 µl of the stop solution (1X - ready to use) to each well, trying to follow the same order in which the substrate solution was added.
7. Absorbance measurement - Measure the optical densities (O.D.) of each well on a microplate spectrophotometer at 450 nm, within 30 minutes of adding the Stop Solution. Before measurement, carefully shake the plate to ensure a homogeneous distribution of the solution.

Technical Support

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For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
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