BMKGENE-Nucleotides sample requirements and Recommendations on tissue samples-Exosomal Transcriptome Sequencing

Recommendations on tissue samples Sample QC can be performed using one of the following four quality assessment methods						
Tissue Type	**Transmission Electron Microscope (TEM)**	**Particle Size and Concentration**	**Flow Cytometry**	**Western Blotting**	**Recommend the total amount of samples**	**Note**
Below are the amounts required for different quality assessment methods for different types of samples						
Exosome	15 μL	15 μL	15 μL	15 μL	100 μL; 40μg	Default exosomal concentration >10^9 particles/ mL
Serum/plasma	0.5 mL	0.5 mL	0.5 mL	1 mL	5 mL	
Cell culture supernatant	10 mL	10 mL	10 mL	20 mL	100 mL	
Urine	10 mL	10 mL	10 mL	20 mL	100 mL	
Saliva	2 mL	2 mL	2 mL	4 mL	15 mL	
Nasal discharge	2 mL	2 mL	2 mL	4 mL	15 mL	
Cerebrospinal fluid	2 mL	2 mL	2 mL	4 mL	15 mL	
Amniotic fluid	5 mL	5 mL	5 mL	10 mL	50 mL	
Semen	0.5 mL	0.5 mL	1 mL	1 mL	5 mL	
Bile	0.5 mL	0.5 mL	1 mL	1 mL	5 mL	
Ascites	5 mL	5 mL	5 mL	10 mL	50 mL	
Milk	2 mL	2 mL	2 mL	4 mL	15 mL	
Follicular fluid	2 mL	2 mL	4 mL	8 mL	15 mL	
Fecal supernatant	5 mL	5 mL	5 mL	10 mL	50 mL	
Microbial culture supernatant	10 mL	10 mL	10 mL	20 mL	100 mL	
Animal tissue	0.3 g	0.3 g	0.5 g	1 g	3 g	
Plant tissue	0.5 g	0.5 g	/	/	1 g	

Nucleotides sample requirements		
Type	**Amount (ng)**	**Volume (μL)**
Total exosomal RNA-NGS	40	20

Recommendations on sample preparation and storage	
Plasma sample (without heparin anticoagulant)	(1) Collect whole blood using a blood collection needle and EDTA anticoagulant tube. Gently invert the tube several times to mix, then store at room temperature or 4 ℃. Proceed to the next step within 1 hour. (2) Under 4 ℃ conditions, centrifuge the tube using a swing-bucket rotor at 1900 g for 10 minutes. Carefully aspirate the supernatant, which is the plasma, and discard approximately 500 μL at the bottom. Centrifuge the obtained plasma again at 4 ℃, 3000 g for 15 minutes. Carefully aspirate the plasma, being careful not to touch the sediment at the bottom or sides of the tube. (3) Freeze the plasma at -80 ℃. Note: It is recommended to provide a sample volume of at least 4 mL (obtaining 4 mL of plasma requires approximately 8-10 mL of whole blood).
Serum sample (Plasma is preferred over serum to avoid the influence of platelet-derived exosome)	(1) Collect 10 mL of blood using a blood collection needle and a regular serum tube (without any additives, 10 mL in size). (2) Let the tube stand at room temperature for 30 minutes, then let it stand for 3-4 hours under 4℃ conditions (during this time, blood clots may form). (3) Use a pipette to aspirate the pale yellow serum from the top (approximately 4 mL) and transfer it to a 15 mL centrifuge tube. Centrifuge at 4 ℃, 3000 g for 15 minutes. Carefully transfer the supernatant to a new 15 mL centrifuge tube, ensuring the highest possible quality of the serum. (4) Freeze the centrifuged serum within 15 minutes at -80 ℃. Note: It is recommended to provide a sample volume of at least 4 mL (obtaining 4 mL of serum requires approximately 10-15 mL of whole blood).
Cell culture supernatant (requires the use of exosome-depleted medium)	(1) Cultivate cells in a regular medium containing serum for a certain period. For adherent cells, the cell density should be around 70%-80% confluence, while for suspension cells, the cell density should be around 60%-70%. (2) For adherent cells, remove the original culture medium and replace it with a new exosome-depleted medium or serum-free medium. For suspension cells, collect the cells by centrifuging at 300 g, 4 ℃ for 10 minutes. (3) Suspend the cells in an exosome-depleted medium or serum-free medium and continue the culture. Continue culturing the cells for 24-48 hours, determining the collection time based on the cell growth rate. (4) Collect the cell culture supernatant by centrifuging at 300 g, 4 ℃ for 10 minutes. Carefully aspirate the supernatant, taking care to avoid aspirating cells or cell debris. (5) Centrifuge the collected supernatant again at 3000 g, 4 ℃ for 15 minutes to ensure the removal of cells or cell debris. (6) Carefully collect the supernatant, avoiding the inclusion of cells or cell debris. Combine the supernatant samples from the same cell culture and transfer them to sterile glass bottles. The supernatant can be stored at 4 ℃ for short-term (1-2 days) or frozen at -80 ℃ for long-term storage. It is recommended to perform exosome isolation as soon as possible after separating the cell culture supernatant, as both 4 ℃ and -80 ℃ storage can have an impact on the yield.

Urine	(1) Collect the first/midstream/last urine sample, preferably fresh urine of 100mL from the subjects. Take care to avoid bacterial contamination. Control the diet before collection. Temporarily store the urine at 4 ℃ (not exceeding 8 hours). (2) Centrifuge the urine at 3000 g for 15 minutes to remove cells or cell debris. (3) Store the urine at -80 ℃ in a freezer.
Cerebrospinal fluid	(1) Collect 10mL of cerebrospinal fluid (CSF) using a lumbar puncture method. Once the sample is separated, please immediately place it on ice or store it at 4 ℃ for a short period (not exceeding 4 hours). Avoid contamination from blood and do not use sample tubes containing heparin anticoagulant. (2) Centrifuge the sample at 3000 g for 15 minutes to remove cells or cell debris. (3) Store the sample at -80 ℃ in a freezer.
Pleural fluid	(1) If unable to process the pleural fluid immediately after clinical collection, please store it temporarily at 4 ℃ (not exceeding 8 hours). (2) Centrifuge the collected pleural fluid at 1000 g for 10 minutes and collect the supernatant. (3) Centrifuge the obtained supernatant of the pleural fluid at 3000 g for 10 minutes and collect the supernatant. (4) Store the collected sample at -80 ℃ in a freezer.
Ascites	(1) If unable to process the ascites fluid immediately after clinical collection, please store it temporarily at 4 ℃ (not exceeding 8 hours). (2) Centrifuge the collected ascites fluid at 2000 g for 20 minutes at 4 ℃ (room temperature is also acceptable) to remove any residue. (3) Store the sample at -80 ℃ in a freezer.
Bile	(1) Collect bile in a sterile container. (2) Centrifuge the collected bile at 4 ℃ and 3000 g for 10 minutes to remove cellular debris and sediment. (3) Collect the supernatant of the bile and store it long-term at 4 ℃ or -20 ℃.
Animal tissue in vitro culture	(1) Upon receiving the tissue sample, culture the sample in the shipment medium to check for contamination. (2) Wash the tissue sample once with RPMI 1640 and use surgical scissors to cut it into 1 mm^3 tissue fragments. (3) Pass the minced tissue fragments through a 70-micron cell strainer to remove tissue debris. (4) Place the tissue fragments into the transwell chambers of a 6-well plate and add RPMI 1640 medium with 10% FBS (free of exosomes). (5) Add 1mL of supernatant to 50mg of tissue and culture for 48 or 72 hours, then collect the culture medium. (6) Centrifuge at 4 ℃, 2000 g for 10 minutes, and collect the supernatant. (7) Centrifuge at 4 ℃, 10000 g for 30 minutes, and collect the supernatant. (8) The supernatant is used for exosome isolation.

Animal tissue enzymatic digestion	(1) Upon receiving the tissue sample, culture the sample in the shipment medium to check for contamination. (2) Discard the supernatant and add 2.4 U/mL of Dispase II. Digest overnight at 4 ℃. (3) Aspirate the Dispase II, rinse twice with PBS, and transfer the tissue sample to a 10 cm cell culture dish containing PBS. (4) Use ophthalmic scissors to cut the tissue sample into approximately 1 mm^3 tissue fragments and place them in a 50 mL centrifuge tube. (5) Add a 0.2% solution of Type I collagenase to the aforementioned centrifuge tube at a volume that is 5 times the tissue volume. Place it in a constant temperature incubator and digest at 37 ℃ with gentle shaking for 1.5 hours until the cell suspension becomes milky. (6) Gently pipette to obtain a single-cell suspension and add PBS to the appropriate volume. (7) Centrifuge at 4 ℃, 2000 g for 10 minutes, and collect the supernatant. (8) Centrifuge at 4 ℃, 10000 g for 30 minutes, and collect the supernatant. (9) The supernatant is used for exosome isolation.
Plant tissue juicing	(1) Clean the sample with distilled water. (2) Use filter paper to remove surface moisture. Perform a 1 minute disinfection with 75% alcohol in a clean bench. (3) Juice the sample using a juicer after peeling (if the juice yield is low, dilute with an appropriate amount of PBS). (4) Transfer the sample to a new centrifuge tube and centrifuge at low speed to collect and save the supernatant.
Plant tissue enzymatic digestion	(1) Take a 5g portion of the sample using a surgical knife, wash it with distilled water, and use filter paper to remove surface moisture. (2) After sterilizing all sampling instruments at high temperature and pressure, perform the following steps in a clean bench: finely chop the sample, soak it in 75% alcohol for 1 minute, rinse off the residual alcohol with distilled water, and dry it with sterile paper. Then, add 20 mL of enzyme solution (4% cellulase, 2% pectinase, 0.6 mol/L mannitol, pH 5.8) and perform enzymatic digestion at 50℃ for 6 hours. (3) Centrifuge the sample at 10,000 rpm for 1 hour to allow the enzymatically digested tissue debris to settle, pass the supernatant through a cell strainer, and collect the filtrate. (4) Take the filtrate and dialyze it overnight using a 300 KD dialysis bag with 1× PBS as the dialysis solution.

Note:

● The collected samples should meet the inclusion criteria, such as age, obesity (height, weight), blood glucose, blood lipids, blood pressure, diagnostic conditions, etc.

● Collect blood samples in the morning on an empty stomach to minimize inter-sample differences.

● Discard samples that experience hemolysis.

● Clearly write the sample numbers on the tube walls and caps using an oil-based pen.

● Seal the centrifuge tubes with sealing film before placing them in the refrigerator.

● If providing frozen cell lines, detailed revival methods should be provided.

● Cell culture medium must use serum-free medium or exosome-depleted serum (such as Thermo Fisher's SFM) to remove exosomes.

● No RNA protectants (such as Trizol) should be added to the samples before isolating exosomes.

● If exosomes are to be observed by transmission electron microscope, they should be stored at 4 ℃ and not kept for too long.

● Whole blood is recommended to be stored in PAXgene tubes, and should not be freeze-thawed. Prepare plasma or serum as soon as possible after blood collection. Plasma or serum can be stored at -80 ℃, but avoid repeated freeze-thaw cycles.

● Storage conditions and storage time affect the yield of exosomes. If samples are stored in a -80 ℃ freezer for too long, the yield of extracellular vesicles will significantly decrease.