TransExo® PS Exosome Isolation Kit

Please read the manual carefully before use

Catalog No. FE601

Storage: At 2-8℃ for one year

Description

TransExo® PS Exosome Isolation Kit utilizes magnetic beads as the core material, which, through the action of phosphatidylserine (PS) affinity receptor proteins, selectively captures exosomes from various samples such as serum, heparin plasma, urine, and cell culture supernatants. Subsequently, under gentle conditions, exosomes are eluted from the magnetic beads using a neutral elution solution, yielding well-shaped, structurally intact, and high-purity exosomes. The extracted exosomes are suitable for various analyses including Western Blot, qPCR, electron microscopy, nanoparticle tracking, and can also be used for cell co-culturing.

Feature

• Compared to ultracentrifugation, it offers stronger specificity and higher purity.

• The elution conditions are gentle, resulting in well-shaped, structurally intact, and highly active extracted exosomes.

• Simple operation, no need for ultracentrifugation, enabling exosome purification to be completed within 3 hours.

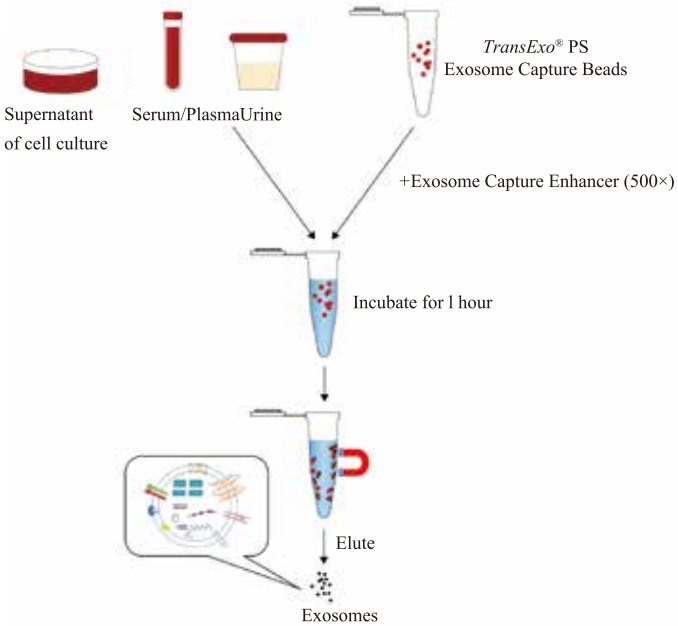
Application

Serum, heparin plasma, cell culture supernatants, urine, etc.

Kit content

Component	FE601-01 (20 rxns)
TransExo® PS Exosome Capture Beads	1 ml
Wash Buffer (10×)	10 ml
Exosome Capture Enhancer (500×)	300 μl
Elution Buffer (10×)	500 μl
1.5 ml Reaction Tube	20 tubes

Operation Process Diagram



Protocol

Self-prepared reagents

Product Name	Catalog
PBS (1×)	TransGen, Cat. FG701-01
100 K Ultrafiltration centrifuge tube	PALL, Cat. MAP100C36

Magnetic stand that are compatible with 1.5 ml centrifuge tubes

Low-temperature high-speed centrifuge (Max > 10,000×g)

Rotary mixer

0.22 μm filter

1. Sample preparation

(1) Serum / Heparin Plasma / Cell Culture Supernatant:

1. Take fresh or -80°C stored serum / heparin plasma / cell culture supernatant samples, centrifuge at 300×g at 2-8°C for 5 minutes to remove cells.
2. Carefully transfer the supernatant to a new centrifuge tube, centrifuge at 2000×g at 2-8°C for 10 minutes to remove cellular debris.
3. Carefully transfer the supernatant to a new centrifuge tube, centrifuge at 10,000×g at 2-8°C for 30 minutes to remove large-sized vesicles.
4. Transfer the supernatant again to a new centrifuge tube and filter the supernatant through a 0.22 μm filter.

   Note: When extracting exosomes from cell culture supernatant, use serum-free medium or exosome-depleted medium for cell culture, and collect the cell culture supernatant after 12-72 hours. It is recommended to concentrate the filtered cell culture supernatant, for example, using a 100 K ultrafiltration centrifuge tube to concentrate 50 ml of cell culture supernatant to 1 ml.

(2) Urine

1. Take fresh or -80°C stored urine samples, centrifuge at 300×g at room temperature for 5 minutes to remove cells.
2. Carefully transfer the supernatant to a new centrifuge tube, centrifuge at 2000×g at room temperature for 10 minutes to remove cellular debris;
3. Carefully transfer the supernatant to a new centrifuge tube, centrifuge at 10,000×g at room temperature for 30 minutes to remove large-sized vesicles;
4. Transfer the supernatant again to a new centrifuge tube and filter the supernatant through a 0.22 μm filter.

   Note: Urine samples may become turbid after thawing from -80°C and need to be shaken gently to restore clarity. Urine sample centrifugation should be performed at room temperature. It is recommended to concentrate the filtered urine, for example, using a 100 K ultrafiltration centrifuge tube to concentrate 50 ml of urine to 1 ml.

2. Buffer Preparation

(1) Prepare Wash Buffer (1×): Dilute Wash Buffer (10×) with ddH2O, and add Exosome Capture Enhancer (500×) to achieve a working concentration of 1×. Prepare the working solution of 3 ml Wash Buffer (1×) per sample, with the volume added as shown in the table below. Store the solution at 2-8°C after preparation and use on the same day.

Component	Volume
Wash Buffer (10×)	300 μl
Exosome Capture Enhancer (500×)	6 μl
ddH_2O	2.7 ml
Total	3 ml

(2) Prepare Elution Buffer (1×): Dilute Elution Buffer (10×) with PBS (1×) to achieve a working concentration of 1×. Prepare the working solution of 150 µl Elution Buffer (1×) per sample, with the volume added as shown in the table below. Store the solution at 2-8°C after preparation and use on the same day.

Component	Volume
Elution Buffer (10×)	15 µl
PBS	135 µl
Total	150 µl

3. Exosome extraction

(1) Take out the *TransExo*® PS Exosome Capture Beads from the kit and gently vortex to mix. For example, for a 500 µl serum sample, pipette 50 µl of beads into a 1.5 ml Reaction Tube.

(2) Add 500 µl of Wash Buffer (1×) to the beads, mix by vortexing, and then place the tube on a magnetic stand for 1 minute until the beads are attracted to the tube wall and the solution becomes clear. Carefully remove the supernatant.

(3) Remove the Reaction Tube from the magnetic stand, add another 500 µl of Wash Buffer (1×) to the beads, mix by vortexing, and then place the tube on the magnetic stand for 1 minute until the beads are attracted to the tube wall and the solution becomes clear. Carefully remove the supernatant.

(4) Add 500 µl of prepared serum sample to the washed beads, along with 1 µl of Exosome Capture Enhancer (500×), and thoroughly mix by pipetting.

Sample	*TransExo*® PS Exosome Capture Beads	Exosome Capture Enhancer (500×)
500 µl serum/heparin plasma	50 µl	1 µl
1 ml concentrated supernatant of cell culture or urine	50 µl	2 µl

Note: When incubating with a 1.5 ml Reaction Tube, the optimal sample volume per tube is between 500 µl and 1 ml.

(5) Place the Reaction Tube in a rotary mixer and incubate at 2-8°C or room temperature for 1 hour.

Note: When extracting exosomes from urine, incubation must be performed at room temperature.

4. Exosome Elution

(1) Place the incubated Reaction Tube on the magnetic stand for 1 minute until the beads are completely absorbed to the tube wall, then carefully remove the supernatant.

(2) Remove the Reaction Tube from the magnetic stand, add 1 ml of Wash Buffer (1×), gently pipette a few times, then place on the magnetic stand for 1 minute until the beads are attracted to the tube wall and the solution becomes clear. Carefully remove the supernatant.

(3) Repeat step (2) to perform the second wash of the beads.

(4) Remove the Reaction Tube from the magnetic stand, add 75 µl of Elution Buffer (1×), gently pipette for 2 minutes for elution.

(5) Place the Reaction Tube on the magnetic stand for 1 minute, carefully transfer the supernatant to a new centrifuge tube.

(6) Add another 75 µl of Elution Buffer (1×) to the Reaction Tube, gently pipette for 2 minutes for the second elution.

(7) Place the Reaction Tube on the magnetic stand for 1 minute, carefully remove the supernatant, mix it with the sample obtained from the first elution. The first and second elution are the purified exosome product.

(8) Purified exosomes can be stored at 2-8°C short term or aliquoted and stored at -80°C to avoid repeated freeze-thaw cycles.

Note: For plasma/serum samples, using 2× Elution Buffer for elution will yield higher elution efficiency. To obtain a higher concentration of exosomes, reduce the amount of Elution Buffer used and extend the elution time accordingly. If extracted exosomes are used for RNA extraction, it is recommended to reduce the amount of Elution Buffer used and extend the elution time to avoid excessive elution buffer affecting RNA extraction efficiency.

Notes

- Reagents should be stored at 2-8°C, do not freeze.
- *TransExo*® PS Exosome Capture Beads should not be centrifuged at high speed for a long time if adhered to the tube cap; use low-speed centrifugation only. It is recommended to use low-adsorption tips when pipetting beads to avoid bead loss.
- Fresh samples are recommended for exosome extraction if further nanoparticle tracking analysis or electron microscopy is conducted. It is also recommended to perform a second separation using magnetic beads on the final exosome solution. Residual beads must be removed.
- Heparin is recommended for plasma samples. This kit is not recommended for exosome extraction from EDTA plasma and citric acid plasma.
- Wash Buffer (1×) should be prepared fresh and used immediately; long-term storage may result in precipitation, affecting its effectiveness.
- For samples with high exosome content, incubation time can be extended appropriately to obtain a higher yield.

For research use only, not for clinical diagnosis.

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