TOOLSharp Plasma Exosome Extraction Kit

For exosome extraction from plasma

Contents

Introduction

The TOOLSharp Plasma Exosome Extraction Kit is specially designed for isolating the exosome, which contains the RNA and protein secreted by various types of cells, from plasma. Compared with traditional ultrahigh-speed centrifugation, the simple low-speed centrifugation used in the aforementioned kit causes less centrifugal stress on the exosome; thus, the morphology of the exosome remains more intact when using low-speed centrifugation than when using ultrahigh-speed centrifugation. Moreover, this product can save time, requires a small number of input samples, and provides high isolation efficiency. The exosomes obtained using this product can be used in various downstream applications, such as protein research, RNA analysis, and high-throughput sequencing.

Kit Contents

Contents	TTC-RF03
Plasma exosome extraction reagent	10 mL
Proteinase K (20 mg/mL)	2 × 900 μL

Output

A total of 5–20 ng of total RNA is extracted using the following protocol: obtain 1 mL of fresh plasma (or unthawed frozen plasma stored at −70 °C), use the plasma exosome extraction reagent to isolate the exosome, and perform RNA isolation.

Storage

The TOOLSharp Plasma Exosome Extraction Kit should be stored at 2–8 °C.

Proteinase K should be stored at room temperature.

Protocol

1. Plasma preparation
   (a) Add a certain amount of anticoagulant into a blood collection tube before collecting blood. Then, add blood into the tube, gently invert the tube 10–15 times to mix thoroughly, set the whole-blood sample still at room temperature for 1 h or at 2–8 °C overnight until the blood coagulates.
   (b) Centrifuge the aforementioned mixture at 4 °C and 1000–2000 × g for 5–10 min, and gently aspirate the supernatant (plasma, half-transparent yellow liquid at the upper layer) into a new centrifuge tube without disturbing the cell component.
   (c) The collected plasma can be directly used in downstream experiments or stored at −70 °C after the splitting is packed.
2. Sample preparation
   (a) Place the fresh plasma on ice. If the sample is frozen, thaw it in a 25 °C water bath until it completely becomes liquid, and then place it on ice.
   (b) Centrifuge the plasma at room temperature for 20 min and at 2000 × g to remove cells and debris.
   (c) Gently aspirate the supernatant into a new centrifuge tube without disturbing the sediment.
   (d) Centrifuge the supernatant at room temperature and at 10 000 × g for 20 min to remove residual debris.
   (e) Gently aspirate the supernatant into a new centrifuge tube without disturbing the sediment and residual liquid, and place the supernatant on ice until use.
3. Exosome extraction
   (a) Transfer the required volume of plasma sample into a new centrifuge tube, add 1× PBS with half the volume of the plasma, and vortex into the centrifuge tube.

Note: (Optional) Because a large amount of protein is contained in plasma, if the to-be-extracted exosome will not be used for protein research, add 0.05 mL of Proteinase K to the aforementioned tube, and incubate the tube at 37 °C for 10 min after vortex mixing.

   (b) Plasma exosome extraction reagent with one-fifth the combined volume of the plasma and PBS is added to the aforementioned tube.

For example:

Plasma volume	1× PBS volume	Optional (Proteinase K)	Reagent volume to be added
1 mL	500 μL	50 μL	300 μL
2 mL	1 mL	100 μL	600 μL

(c) Gently invert the solution or pipette it up and down several times to mix it well until a homogenous solution is obtained.

Note: The solution should be cloudy.

(d) Stand the mixture at 2–8 °C for 30 min for incubation.

(e) Centrifuge the mixture at room temperature and 10 000 × g for 5 min.

(f) Carefully discard the supernatant by using a pipette.

(g) Centrifuge the obtained solution at room temperature and 10 000 × g for 30 sec, and discard the residual liquid by using a pipette to obtain the exosome contained in the sediment.

(h) Resuspend the exosome sediment by using 1× PBS, or directly apply the exosome sediment in the subsequent experiments.

Note: If necessary, store the exosome at 2–8 °C for up to 1 week or at −20 or −70 °C for a long period.

Troubleshooting

♦ What sample volume is required for a single experiment?

A plasma volume of at least 0.5 mL is required because a lower plasma volume will fail to extract a sufficient amount of exosome to meet the experiment demands.

♦ Why is Proteinase K added to the solution when extracting exosomes from plasma?

Plasma contains a large amount of protein, such as fibrinogen, which is a type of coagulant that may have undesirable effects on the experiments conducted with the exosomes. Therefore, if the to-be-extracted exosomes will not be used for protein research, Proteinase K can be used to digest unwanted protein before extraction.

However, Proteinase K can also digest the surface proteins of the exosomes.

♦ How can the exosome sediment be resuspended?

The isolated exosome sediment can be resuspended using 1× PBS or the reagent used in the downstream experiment. If the downstream experiment does not require the exosomes to remain intact, a low-speed homogenizer can be used in the resuspension procedure.

♦ Other important points

The blood component is complex. For some difficult-to-isolate samples, such as hyperlipoidemia samples, exosome extraction and subsequent treatment should be adjusted according to the actual situation.