

# VEX Exosome Isolation Reagent (from plasma)

R603



Version 8.1

Vazyme biotech co., Ltd.

## Introduction

VEX Exosome Isolation Reagent (from plasma) is specially designed for isolating the exosome, containing RNA and protein secreted by various types of cells, from plasma. Compared with the traditional ultra-speed centrifugation, the simple low-speed centrifugation involved in this reagent makes the exosome less affected by centrifugal stress, therefore more intact in morphology. Meanwhile, VEX Exosome Isolation Reagent can save the experiment time, requires less input amount of sample, and with high isolation efficiency. The exosomes obtained by this product can be applied to a variety of downstream applications, such as protein research, RNA analysis, high-throughput sequencing, etc.

## Components

Components	R603
VEX Exosome Isolation Reagent (from plasma)	10 ml
Proteinase K (20 mg/ml)	2 X 900 $\mu$ l

## Storage

VEX Exosome Isolation Reagent (from plasma) should be stored at 2 - 8°C, transported at 2 - 8°C or room temperature. Proteinase K should be stored at -15 ~ -30°C, transported at -20 - 4°C.

## Quality Control

Function assay: obtain 1 ml of fresh plasma (or frozen plasma stored at -70°C, never thawed before), use VEX Exosome Isolation Reagent (from plasma) to isolate exosome, from which followed by a RNA isolation, and lastly, take 2 ng - 5 ng of that RNA as template to detect expressions of two small RNA by qPCR.

## Workflow

### 1. Plasma preparation

- (1) Add a certain proportion of anticoagulant into a blood collection tube before blood collecting, collect blood into the tube, gently invert the tube 10 - 15 times to mix thoroughly, set this whole blood still at room temperature for 1 hour or at 2 - 8°C overnight until blood coagulated.
- (2) Centrifuge at 4°C, 1000 - 2,000 x g for 5 - 10 mins, gently aspirate the supernatant (plasma, half-transparent yellow liquid at the upper (3) layer) into a new centrifuge tube without disturbing the cell component.
- (3) This collected plasma can be directly applied to downstream experiments, or stored at -70°C after splitting packed.

### 2. Sample preparation

- (1) Place the fresh plasma on ice. If the sample is frozen, please thaw in 25°C water bath until it totally becomes liquid, and place it on ice.
- (2) Centrifuge at room temperature for 20 mins, 2,000 x g to remove cells and debris.
- (3) Gently aspirate the supernatant into a new centrifuge tube without disturbing the sediment.
- (4) Centrifuge at room temperature, 10,000 x g for 20 mins to remove residual debris.
- (5) Gently aspirate the supernatant into a new centrifuge tube without disturbing the sediment and residual liquid, place on ice till use.

### 3. Exosome extraction

- (1) Transfer a required volume of plasma sample into a new centrifuge tube and add 1/2 volumes of 1 × PBS, vortex to mix.  
(Optional) Since a great amount of protein is contained in plasma, if the to-be-extracted exosome will not be used for protein research, please add 0.05 volume of Proteinase K to the above tube and incubate at 37°C for 10 min after vortex mixing.



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(2) Add 1/5 total volumes (plasma + PBS) of VEX Exosome Isolation Reagent (from plasma).

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

(3) Gently invert or pipette up and down for several times to mix well until there is a homogenous solution.

\* The solution should be cloudy.

(4) Stand the mixture at 2 - 8°C for 30 mins for incubation.

(5) Centrifuge at room temperature, 10,000 x g for 5 mins.

(6) Carefully discard the supernatant by pipette.

(7) Centrifuge at room temperature, 10,000 x g for 30 seconds, discard the residual liquid by pipette to obtain the exosome contained in sediment.

(8) Resuspend the exosome sediment by 1 X PBS or directly apply to subsequent experiment.

\* If necessary, store the exosome at 2 - 8°C for up to 1 week, or -20°C/-70°C for long term.

## Troubleshooting

### ◇ How much sample volume does a single experiment need?

At least 0.5 ml, because the plasma volume less than that will fail to extract sufficient exosome to meet the following experiment demands.

### ◇ Why adding Proteinase K when extracting exosome from plasma?

A great amount of protein is contained in plasma, such as fibrinogen, a kind of coagulation factor that may have unnecessary effects on subsequent experiments by exosome. Therefore, if the to-be-extracted exosome will not be used for protein research, the Proteinase K can be used to digest unwanted protein before extraction. But one thing need to notice is that the Proteinase K can digest the surface protein of exosome as well.

### ◇ How to resuspend the exosome sediment?

The isolated exosome sediment can be resuspend by 1 X PBS or directly by the reagent subsequently used in the downstream experiment. If downstream experiment do not require exosome to remain intact, low speed homogenizer could be used in resuspension procedure.

### ◇ Other notices

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



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