Minute™ Hi-Efficiency Exosome Isolation Reagent

Cat. No. EI-027

Description

Exosomes are vesicles secreted by cells. They are present in a variety of body fluids such as serum, ascites, spinal cord fluid, urine, and saliva. Cultured cells also secrete significant number of exosomes. The size distribution of exosome is ranging from 30-120 nm. The biological function of exosomes is believed to serve as intercellular messengers. Filtration and ultracentrifugation are classical ways of isolating and enriching exosomes. This is a tedious process and requires special equipment. Another common way for enrichment of exosome is through precipitation. Currently major commercial kits are PEG-based. EI-027 is designed to precipitate total exosomes from biofluids and cell culture medium using a high efficacy, non-PEG based reagent for exosome precipitation. Unlike many other companies that require a specific kit for a specific type of sample, this kit can be applied to most commonly used biofluid samples using the same reagent and similar protocol.

Packaging: Exosome Precipitation Reagent 20 ml

Shipping and Storage: This product is shipped and stored at ambient temperature

Important Notes:

A.
This kit can be used for enrichment of total exosomes from samples such as serum, ascites, plasma, cell culture medium, spinal cord fluid, saliva and urine. However, there are some variations in specific steps for sample preparation, sample pre-treatment, and centrifugation force used for each specific sample. Following protocol is a general one for all samples. The table below specify specific method and g force recommended for different samples.

B.
For exosome isolation from cultured cells, to make sure the exosomes come from your cells of interest, exosome depleted fetal bovine serum should be used for cell culture. If this not possible, cells should be harvested, washed at least 2 times with PBS and cultured in serum-free medium for up to 15h. After incubation, the medium can be separated from cells by low speed centrifugation and used for exosome isolation.

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Protocol

1. Place your sample in a test tube and centrifuge at 2000 X g for 10 min to remove large debris. See table below for pre-treatment prior to step 1.
2. Transfer the supernatant to a fresh tube and add ½ volume of exosome precipitation reagent to the tube (for example add 50 µl reagent to 100 µl sample)
3. After incubation, centrifuge the tube at 4°C for a period of time specified in table 1 below. Remove the supernatant and spin the tube at 10,000 X g for 30 seconds to 1 min to bring down liquid that may adhere to the wall of the tube. Carefully remove the residue liquid in the tube completely. Resuspend the pellet in1 X PBS (pH 7.2-7.4) or other buffer of your choice. The amount of buffer used depends on the size of the pellet (for serum sample, the amount of resuspension buffer is about 1-2 volumes of starting sample volume). In some cases, precipitated exosomes are not visible and could be attached to side wall of a test tube. Be sure to wash the wall of the tube with resuspension buffer if the exosome pellet is not visible. Resuspended exosome is now ready for downstream experiments such as isolation of RNA and Western blot and other analysis.

Table 1. Experimental conditions for different samples

Sample types	Pre-treatment	Volume	Incubation Time step 2	X g in step 3
Serum	No	>10 µl	30 min-1h	10,000 X g 15 min
Plasma	Dilute 1:2 with PBS	>10 µl	30 min-1h	10,000X g 15 min
Ascites	No	>50 µl	30 min-1h	10,000X g 15 min
Cell culture medium	See note B above	>1 ml	overnight	10,000 X g 1h
Urine and spinal cord fluid	No	>1 ml	overnight	10,000 X g 1h

Note: Plasma contains significant amount of blood coagulation related proteins **that may** interfere with exosome precipitation**.** An alternate is to treat plasma sample with proteinase K but this may result in partial loss of exosome surface proteins.

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