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. 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 serves a general guide 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.
C.
Since the concentration of exosomes in cell culture supernatant and spinal cord fluid is usually significantly lower than that of serum and tissue extract, it is recommended to add small amount of BSA as a carrier to these samples prior to precipitation.

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Protocol

Important: Shake the reagent bottle a few times to mix the contents well prior to use

1. Place your sample in a test tube and centrifuge at 2,000 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). Mix well by vortexing briefly.
3. After incubation, centrifuge 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. Add 100-200 ul 1 X PBS (pH 7.2-7.4) to the tube and remove immediately to remove the residue reagent and soluble proteins. Resuspend the pellet in 1X PBS 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.

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**	Add BSA see below	>1 ml	1h-overnight	12,000 X g 30 min-1h
Urine	No	>1 ml	overnight	14,000 X g 1h
Spinal cord fluid**	Add BSA see below	>1 ml	1h-overnight	12,000 X g 30 min-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.

** Prepare 5% BSA solution in deionized H_2O. Dilute BSA with tissue culture supernatant or spinal cord fluid 1:10, e.g. mix 100 µl 5% BSA with 900 µl sample. The final BSA concentration is 0.5%.

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