PRODUCT INFORMATION

Exo-Max Exosome Purification Kit

v. 230201

Catalog number	C08010-K01
Package	10 rxns
Description	Exosomes and other extracellular vesicles (EVs) are small membrane vesicles containing protein, mRNA, microRNA, DNA, and lipids, which are secreted by various cells and are table in body fluids including blood, urine, saliva etc. These EVs are recognized as biomarkers for many diseases. Exo-Max Exosome Purification Kit provides an innovative affinity purification method using recombinant protein conjugated magnetic beads, which can specifically capture EVs. This kit can isolate high purity EVs from cell culture medium and body fluids following a 10,000 x g centrifugation. This kit also enables the isolation of intact form of EVs by eluting EVs from magnetic beads with attached reagent. The isolated intact exosomes and other EVs can be used for various applications including electron microscopic analysis, nanoparticle tracking analysis and other protein and molecular analysis such as Western blotting and sequencing.
Kit component	See table below
Product capacity	The binding and elution capacity of 20 µL Exosome Purification Beads are commonly equal to 10-200 µg of exosomes or EVs.
Materials and reagent required but not provided	• Micro-centrifuge capable of <10,000 x g • 1.5 mL Centrifuge tubes • 15 mL Centrifuge tubes • 50 mL Centrifuge tubes • Vortex mixer • Micro pipette • Pipette tip • End-over-end rotator • Syringe Filter Unit, 0.22 µm, PVDF • Ultrafiltration unit (e.g. Cut-off 100 K of Vivaspin or Amicon® Ultra Centrifugal

Reagent	Quantity for 10 rxns
Exo-Max Exosome Purification Beads	0.2 mL X 1 vial
Binding Buffer	10 mL X 1 vial
Washing Buffer	20 mL X 1 vial
Elution buffer	1 mL X 2 vial
Exo-Cryo Media	0.5 mL X 1 vial

Note: Exosome Purification Beads is 1:1 suspension in Phosphate Buffered Saline, pH 7.4, containing 0.05% sodium azide as a preservative.

	Filter Units) • Magnetic stand • TBS buffer
Storage	2 °C to 8 °C. **DO NOT FREEZE.**
Precautions **Disclaimer**	This product is for research use only and is not intended for diagnostic use.
Procedure	General notes The Exosome Purification Beads is stored in Phosphate Buffered Saline containing 0.05% sodium azide. The storage buffer (PBS) must be removed before use and the magnetic beads should be equilibrated with Binding Buffer. The equilibration can be performed at room temperature or at 2-8 °C. When using cell culture samples contain animal serum (e.g. FBS), **exosome-depleted serum** is general recommend for experiments Sample preparation **A. Preparation of sample from cell culture medium** 1. Harvest cell culture medium by centrifuging at 300 x g for 10 minutes at 4 °C. 2. Transfer the supernatant into a new tube. 3. Centrifuge at 2000 x g for 30 minutes at 4 °C to remove the rest of cell debris. 4. Transfer the supernatant into a new tube. 5. Centrifuge at 10,000 x g for 30 minutes at 4 °C and using 0.22 µm PVDF Syringe Filter Unit to remove the large EVs. 6. Transfer the supernatant into a new tube. The sample should keep around 1 mL of volume for further step. Note: Since conditioned medium usually in a large volume, it recommends to concentrate samples using Ultrafiltration unit as mentioned above. **B. Preparation of sample from serum and plasma** 1. Centrifuge at 2000 x g for 30 minutes at 4 °C and transfer the sample into a new tube. 2. Centrifuge at 10,000 x g for 30 minutes at 4 °C and using 0.22 µm PVDF Syringe Filter Unit to remove the large EVs. 3. Transfer the supernatant into a new tube. Usually, serum or plasma volume can be used around 50~250 µL per reaction.

Procedure	Affinity reaction 1. Add Exosome Purification Beads (20 µL) in 1 mL sample (dilute in Binding Buffer if necessary) and react for 0.5-3 hours at 2-10 °C with rotator. 2. Remove the supernatant using Magnetic stand. 3. Add 1 mL Washing Buffer into the tube, then vortex gently and stand for 1 minute. Remove the waste using Magnetic stand. 4. Repeat step 3 twice. 5. Add 50 µL Elution Buffer and resuspend beads by tapping the bottom of tube and stand for 10 minutes at room temperature. Option: Beads can be used for further experiments. For Western blotting, loading buffer can be added and perform denaturing procedure directly. For nucleic acid extraction, lysis buffer can be added and perform the following extraction steps. 6.Isolate beads using Magnetic stand and transfer exosome-contained supernatant into new tube. The sample is ready to the further experiment. For long term storage, 4-fold volume of **Exo-Cryo Media** can be mixed with samples and storage at -80 °C until use.

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