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Extracellular Vesicle Isolation Kit

Item No. 702420

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage Temperature
400529	Extracellular Vesicle Isolation Reagent	1 vial/6 ml	4°C
400535	Extracellular Vesicle Storage Medium	1 vial/20 ml	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Microcentrifuge tubes (1.5 ml)
2. Refrigerated centrifuge capable of 15,000 x g
3. PBS

INTRODUCTION

Background

Extracellular vesicles, such as exosomes, are 30-150 nm vesicles released from cells that contain heterogeneous mixtures of proteins, nucleic acids, lipids, and sugars and are found in a variety of bodily fluids, including plasma, serum, and urine.[1,2] Exosomes are formed by inward budding of endosomes, stored as intraluminal vesicles in multi-vesicular bodies, and released *via* fusion of the multi-vesicular bodies with the plasma membrane.[1] Exosomes have various functions, such as remodeling the plasma membrane to remove certain proteins and acting as intercellular messengers. For example, during reticulocyte maturation, transferrin receptors are removed *via* exosomes, in T cells miRNA is packaged and released in exosomes to modulate gene expression in recipient cells reducing Th1 cell proliferation and inflammation, and cancer cells release miRNA in exosomes to modulate the tumor microenvironment.[1,3,4] Because exosomes mirror the parent cell in their contents and outer membrane composition, it is possible they, or the mechanisms of their biogenesis and release, could be used as non-invasive biomarkers for the diagnosis, prognosis, and treatment of diseases.

About This Assay

Cayman's Extracellular Vesicle Isolation Kit provides a simple, quick, and convenient precipitation-based method for isolating extracellular vesicles from serum and plasma. The volume of Extracellular Vesicle Isolation Reagent provided is sufficient for processing up to 24 ml of serum or plasma, and it enables rapid isolation within one hour. Also provided is a storage medium for stabilizing extracellular vesicles during frozen storage. This optional reagent will not interfere with protein assays and is formulated to be compatible with typical downstream analyses.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Extracellular Vesicle Isolation Reagent**

   This reagent is ready to use as supplied.

1. **Extracellular Vesicle Storage Medium**

   This reagent is ready to use as supplied. Thaw immediately prior to use and briefly vortex to ensure any crystalline salts return into solution.

Sample Matrix Properties

The Extracellular Vesicle Isolation Reagent can be used to isolate extracellular vesicles from serum, EDTA plasma, or heparin plasma samples. Extracellular vesicles may be prepared from freshly collected blood or previously frozen serum or plasma.

We recommend storing serum and plasma at -80°C prior to isolation of extracellular vesicles to ensure effective preservation of vesicle cargo for downstream applications. Avoid multiple freeze-thaw cycles of plasma or serum prior to isolation to ensure extracellular vesicles can be effectively resuspended.

Extracellular vesicle fractions prepared from plasma samples may require longer resuspension times, due to co-precipitation of clotting factors. Average particle size and the degree of exosome marker enrichment are, however, unaffected by the presence of anticoagulants and these samples can be resuspended effectively. (see **Performance Characteristics**, on page 8).

The Extracellular Vesicle Isolation Reagent is not recommended for isolation of exosomes from cell culture media or bodily fluids other than serum or plasma.

ASSAY PROTOCOL

Performing the Assay

1. Prepare fresh serum or plasma or, if using previously frozen samples, thaw on ice completely.
2. Centrifuge sample at 3,000 x g for 15 minutes at 4°C.
3. Transfer the supernatant to a new tube without disturbing the pellet, which may or may not be visible depending on sample type.
4. Add Extracellular Vesicle Isolation Reagent (Item No. 400529) to the transferred supernatant in a 1:4 (reagent:sample) ratio.

Extracellular Vesicle Isolation Reagent	Serum/Plasma
25 µl	100 µl
250 µl	1 ml

5. Mix sample thoroughly by vortexing or pipetting up and down to ensure there is a homogeneous suspension.
6. Incubate samples at 4°C for 30 minutes.
7. Following incubation, centrifuge the sample at 10,000 x g for 10 minutes at 4°C.
8. Carefully remove and discard the supernatant. The extracellular vesicle pellet will be at the bottom of the tube.
9. Extracellular vesicle pellets may be resuspended in PBS or a buffer appropriate for the next application. For effective vesicle resuspension, resuspending in at least 50% of the initial serum or plasma volume is recommended. Resuspend the pellet by pipetting up and down several times. For optimal long-term storage of extracellular vesicles, resuspending in Cayman's Extracellular Vesicle Storage Medium (Item No. 400535) is recommended.

Performance Characteristics

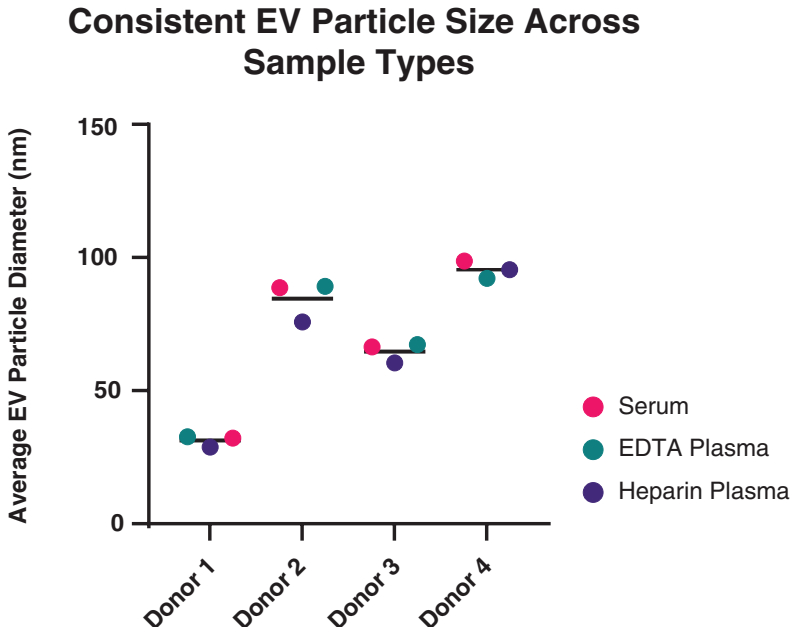

Figure 1. Extracellular Vesicle Isolation Reagent performs consistently across different sample types. Dynamic light scattering, a technique used to determine the size of small particles, was used to assess the average particle size of extracellular vesicles prepared from serum and plasma from matched healthy human donors. Average particle diameter was consistent within each donor across the different sample types tested. Protein recovery, determined *via* Cayman's Protein Determination (BCA) Kit (Item No. 701780), similarly showed a consistent yield for each donor across sample types (data not shown).

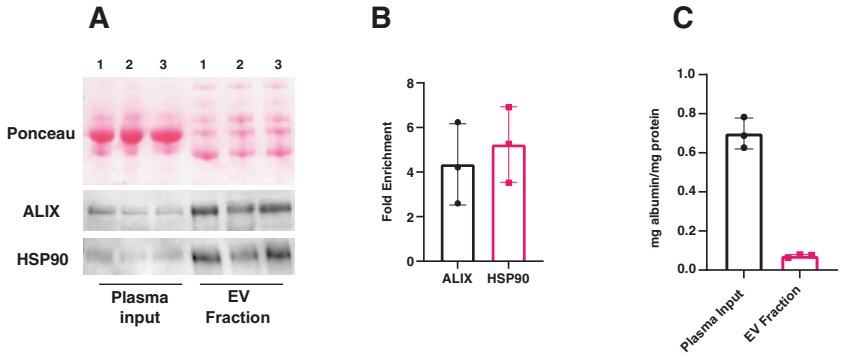

Figure 2. Analysis of exosome markers. Immunoblots for ALG-2-interacting protein (ALIX) and heat-shock protein 90 (Hsp90) were prepared from three different human plasma samples (*Panel A*; 20 µg protein/lane). Extracellular vesicle (EV) fractions exhibited several-fold enrichment compared with plasma input. Non-exosome-associated protein albumin, determined *via* Cayman's Albumin (human) ELISA kit (Item No. 501760), was reduced 10-fold in extracellular vesicles compared with plasma input (*Panel C*).

References

1. Yáñez-Mó, M., Siljander, P.R.-M., Andreu, Z., *et al.* Biological properties of extracellular vesicles and their physiological functions. *J. Extracell. Vesicles* **4**, 27066 (2015).
2. Brennan, K., Martin, K., FitzGerald, S.P., *et al.* A comparison of methods for the isolation and separation of extracellular vesicles from protein and lipid paticles in human serum. *Sci. Rep.* **10(1)**, 1039 (2020).
3. Okoye, I.S., Coomes, S.M., Pelly, V.S., *et al.* MicroRNA-containing T-regulatory-cell-derived exosomes suppress pathogenic T helper 1 cells. *Immunity* **41(1)**, 89-103 (2014).
4. Tomasetti, M., Lee, W., Santarelli, L., *et al.* Exosome-derived microRNAs in cancer metabolism: possible implications in cancer diagnostics and therapy. *Exp. Mol. Med.* **49(1)**, e285 (2017).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.