Extracellular Vesicle Isolation Kit (Tissue Culture)

Item No. 702630

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage Temperature
400609	Extracellular Vesicle Isolation Reagent (Tissue Culture)	1 vial/10 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 10,000 x g

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 (Tissue Culture) provides a simple, quick, and convenient precipitation-based method for isolating extracellular vesicles from tissue culture media. The volume of Extracellular Vesicle Isolation Reagent provided is sufficient for processing up to 40 ml of media. 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 (Tissue Culture)**

   This reagent is ready to use as supplied.

2. **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 (Tissue Culture) can be used to isolate extracellular vesicles from tissue culture media. Extracellular vesicles may be prepared from freshly collected or previously frozen media.

It is recommended to store conditioned media 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 media prior to isolation to ensure extracellular vesicles can be effectively resuspended.

The Extracellular Vesicle Isolation Reagent (Tissue Culture) is not recommended for isolation of exosomes from serum or plasma. Cayman's Extracellular Vesicle Isolation Kit (Plasma and Serum) (Item No. 702420) is optimized for these sample types.

ASSAY PROTOCOL

Performing the Assay

1. Collect fresh conditioned media 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. 400609) to the transferred supernatant in a 1:4 (reagent:sample) ratio.

Extracellular Vesicle Isolation Reagent	Tissue Culture Media
250 µl	1 ml
1 ml	4 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 overnight.
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, which may or may not be visible, 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. 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

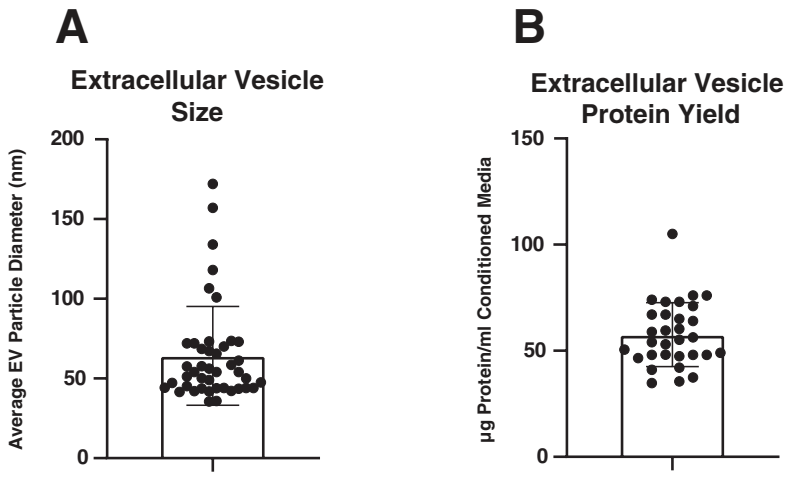

Figure 1. Typical extracellular vesicle (EV) size and protein yield parameters. Dynamic light scattering, a technique used to determine the size of small particles, was used to assess the average size of isolated EVs (*Panel A*). UV/Vis spectroscopy was used to determine the protein concentration of EVs isolated from various sources of conditioned tissue culture media (*Panel B*).

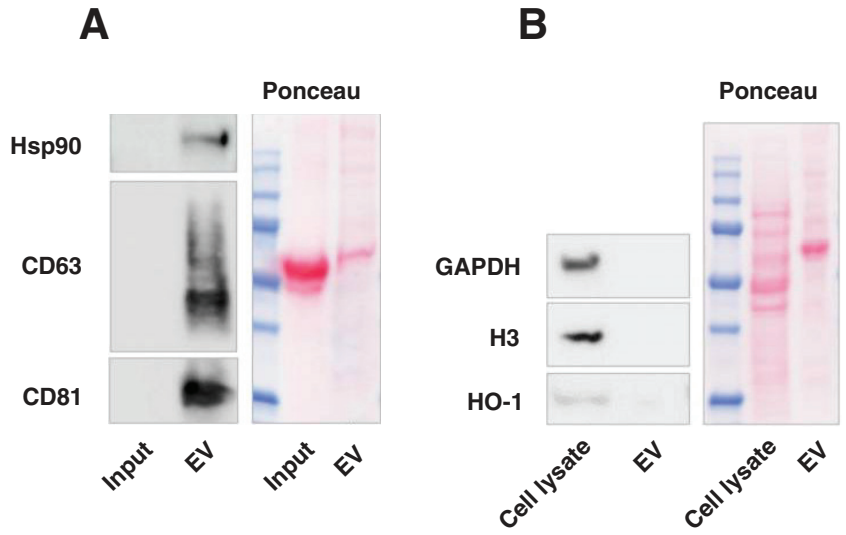

Figure 2. Analysis of exosome markers. Immunoblots were prepared from EV samples isolated from 48 hours-conditioned culture media and compared with input conditioned media (*Panel A*: 15 µg protein/lane). The EV-associated proteins heat shock protein 90 (Hsp90), CD63, and CD81 each exhibited robust enrichment in EV fractions compared with conditioned tissue culture media. When compared with cell lysates (*Panel B:* 15 µg protein/lane), non-EV-associated cytosolic (GAPDH), nuclear (histone H3), and endoplasmic reticulum (HO-1) proteins were not enriched in EV fractions.

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 particles 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.