Product Analysis Certificate

PRODUCT	**Purified Exosome from HT1080, human fibrosarcoma cell line**
CATALOG #s	**EXOP 410A-1**
SIZE	50 ug (>1x10^6 frozen exosomes) (Protein concentration 1µg/µl)
LOT #	**22xxxx-xxx (TBD)**
STORAGE	-20°C
SHELF LIFE	12 months from date of receipt with proper storage
SHIPPING	Dry Ice

PACKAGE CONTENTS:

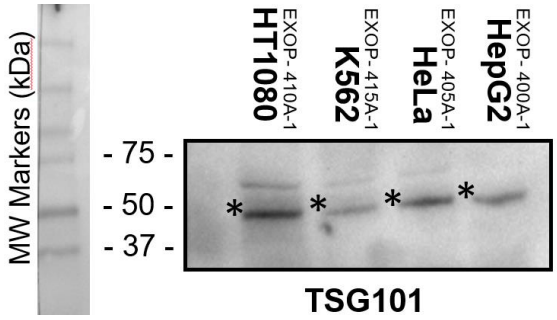
Purified exosome from HT1080 cell line
50 µg exosomes (>1x10^6 frozen exosomes) in 50 µl sterile 1x PBS

DESCRIPTION

Exosomes are 60 - 180 nm membrane vesicles secreted by most cell types in vivo and in vitro and contain distinct subsets of RNAs and proteins depending upon the cell type from which they are secreted, making them useful for biomarker discovery and functional characterization.

- **Cell lines were grown in exosome-depleted FBS (Exo-FBS)**
- **Exosomes purified using ExoQuick-TC**
- **Characterized by NanoSight for size and integrity**
- **Western blot analysis validated**
- **>1x10^6 exosomes (50 µg protein)**

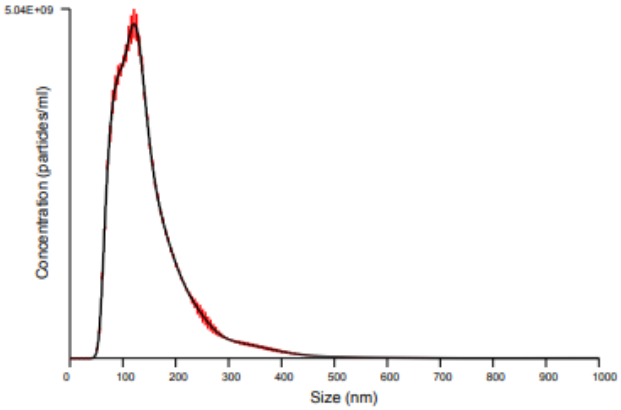
BIOMARKER VALIDATION



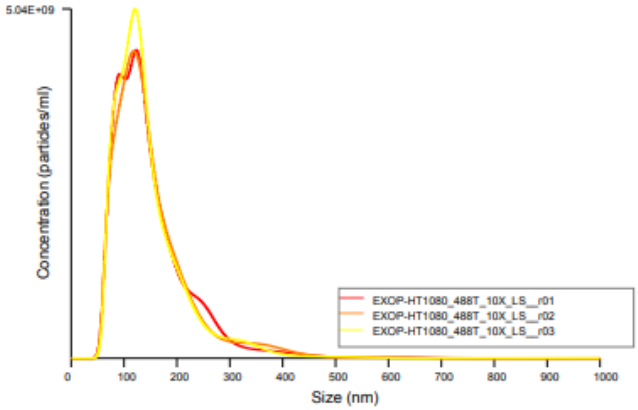
An aliquot of the purified exosomes from the cell lines were lysed with RIPA buffer to make exosomes protein lysates. Approximately 20 ug of protein for each sample was separated on a gradient SDS-PAGE and then transferred to nitrocellulose membranes. The membranes were probed for TSG101 profiles using SBI's anti-TSG101 antibody (cat# EXOAB-TSG101-1) at a 1:1,000 dilution. Bands were detected using the secondary HRP-conjugated antibody at 1:10,000 and blots imaged. All purified exosomes preparations are positive to immunoreactive of TSG101, CD9, CD63, and/or CD81 and exhibit banding patterns common to published exosome biomarkers' profiles.

NANOSIGHT DATA

Approximately 5 ul of the purified exosomes sample was added to 995 ul of 0.2 um filtered 1X PBS (1:200 dilution). The diluted samples were incubated in a VWR 500 model ultrasonicator water bath set at 33°C for 10 minutes to ensure adequate exosome particle dispersion. The samples were diluted 1:10 then vortexed at 2.5k for 10 seconds. This eventual 1:2,000 dilution was used to gather between 1,000 to 3,000 particle tracks per sample analysis. The samples were then loaded into a NanoSight LM10HSB with a syringe pump and the sensitivity of the camera is set to auto 16 (the most sensitive auto-setting). All data were collected in triplicate. The purified exosomes displayed the expected size distribution profiles, with peak diameters between 90-110 nm and concentrations in the range expected for media exosomes at about 1x10^10 exosomes/ml.



FTLA distribution - Average Size / Concentration
Red error bars indicate +/- 1 standard error of the mean



Size / Concentration

Total Concentration:

2.86e+011 +/- 2.26e+009 particles/ml