

## Cellular uptake of fluorescent-labeled exosomes isolated by ultracentrifugation method with ExoCap™ Ultracentrifugation/Storage Booster (ExoCap™ USB)

These data were kindly provided by Kyojiro Kawakami Ph.D, and Masafumi Ito M.D., Ph.D., (Research Team for Mechanism of Aging, Tokyo Metropolitan Institute of Gerontology.)

### Experimental procedures

#### 1. Isolation of exosomes

- Human castration-resistant prostate cancer PC-3 cells were cultured in Advanced RPMI 1640 Medium without FBS for 3 days.
- The cell culture supernatant was centrifuged at 2,000 x g for 10 minutes to eliminate cells followed by centrifugation at 12,000 x g for 30 minutes to discard cell debris.  
Then, the supernatant was filtered through 0.22 μm PVDF membrane.

##### Ultracentrifugation method

- Exosomes were pelleted by ultracentrifugation at 110,000 x g for 70 minutes and washed with PBS.
- After washing, pellets were re-suspended in PBS.

##### Ultracentrifugation method with ExoCap™ USB

- Prior to ultracentrifugation, ExoCap™ USB was added to the filtered sample at a final concentration of 10% according to the manufacturer's instruction.
- Ultracentrifugation was performed as described in the left column. Pellets were washed with and re-suspended in ExoCap™ USB containing buffer.

#### 2. Fluorescent labeling of cultured cells

PC-3 cells were labeled by CellTracker™ Red (Thermo Fisher Scientific Inc.).

#### 3. Fluorescent labeling of isolated exosomes and removal of excess fluorescent dyes

- Six micrograms of isolated exosomes were labeled by PKH67 (Sigma-Aldrich Co. LLC).
- Excess PKH67 that did not bind to exosomes was removed by ultrafiltration using Amicon® Ultra 50 kDa (Merck Millipore Corp.).

For recovery of exosomes from filters, PBS was used for exosomes isolated by **ultracentrifugation** with PBS, while ExoCap™ USB containing buffer was used for exosomes isolated by **ultracentrifugation** with **ExoCap™ USB**.

- The protein concentration and fluorescent intensity were measured in each sample.

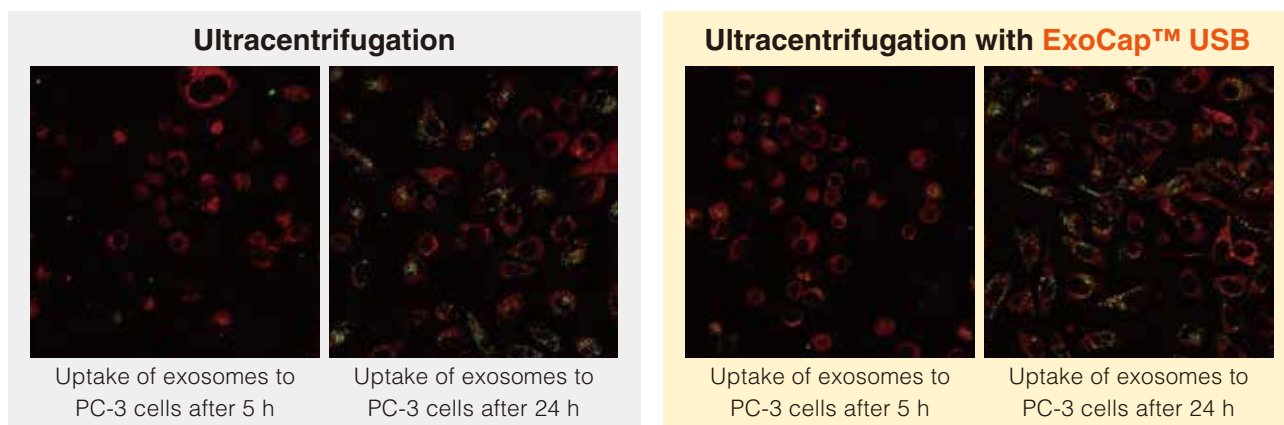
## Results

### Exosome recovery after ultrafiltration

	Ultracentrifugation with PBS	Ultracentrifugation with ExoCap™ USB
Recovery rate of protein (Relative value)	63.6% (1.0)	85.5% (1.3)
Fluorescent intensity (Relative value)	686, 612 (1.0)	1,022,016 (1.5)

### Exosome uptake to cultured PC-3 cells

PKH67-labeled exosomes were added to the culture medium of PC-3 cells at a final concentration of 0.3 µg/100 µL. The cellular uptake of labeled exosomes was examined by fluorescence microscopy.



## Conclusions

- The recovery of exosomes after ultrafiltration was improved by using ExoCap™ USB.
- Exosomes isolated by ultracentrifugation using ExoCap™ USB can be used for PKH67 labeling and cellular uptake experiments.

Code No.	Product Name	Size
MEX-USB	ExoCap™ Ultracentrifugation/Storage Booster	50 mL