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Innovating Epigenetic Solutions

CaptEV reagent

CaptEV: EV pre-enrichment reagent

Cat. No. **C28030001 (cell culture)**

Cat. No. **C28030002 (serum/plasma)**

Version 1 I 12.16

Content

MANUAL

Introduction

CaptEV precipitation reagent has been specifically designed as a pre-enrichment tool prior to extracellular vesicle purification. This reagent is ideally suited for total extracellular vesicle (EV) recovery for one biofluid that can later be processed by immunocapture using Diagenode's ExoIP™ kit.

The CaptEV reagent has been designed to precipitate all vesicles within a single sample to avoid bias. After precipitation, the exosome-rich suspension can be further purified by several means. The reagent also contains chemicals that support downstream immunocapture by facilitating exosome-bead complex formation. These chemicals are simple to wash away after the exosome isolation providing a highly enriched sample. As shown in Figure 1, exosomes are not trapped in sticky polymer and are efficiently isolated due to the CaptEV.

The CaptEV reagent is straightforward to use and allows for reproducibility between experiments.

Advantages of CaptEV:

1. Full recovery of EVs
2. Enhanced immunocapture
3. Better signal coverage in FACS than with other reagents
4. Ease of use
5. Reproducibility
6. Intact and preserved exosomes (not sticky)

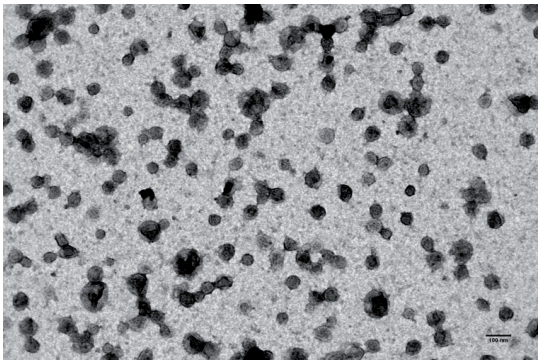
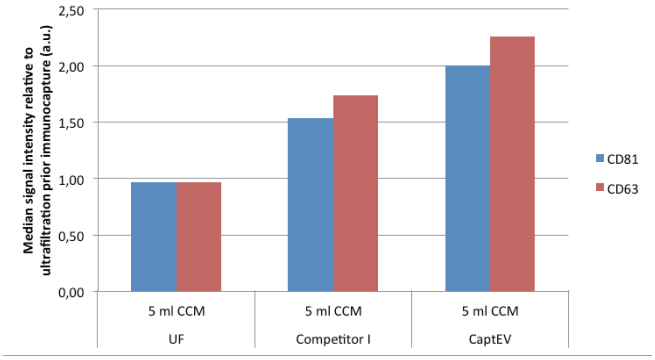


Figure 1: : TEM picture of an exosome preparation obtained with Diagenode ExoIP anti-CD81 kit with elution of beads and Diagenode CaptEV reagent as a pre-enrichment tool



Graph 1: Median signal intensity from the flow cytometry analysis of the exosome capture efficiency corresponding to different pre-enrichment ways prior immunocapture (ExoIP™)

Required materials not provided

- Phosphate Buffered Saline (PBS)
- Pipetus
- Serological pipettes
- Micropipettes and corresponding tips: 20µl – 200µl – 1000µl
- 15ml and/or 50ml tubes for cell culture supernatant collection
- Centrifuge
- Gloves
- Crushed ice

Remarks before starting

CaptEV reagent for cell culture supernatant or for serum and plasma has been designed to be used as a pre-enrichment tool prior to EV purification.

It has been specially designed to work with Diagenode's ExoIP™ immunocapture kits. However, other purification may be used if desired.

Moreover, concerning the cell culture supernatant; this reagent works well with conditioned cell culture supernatant. In normal conditions of use, exosome-depleted FBS complemented cell culture media can be used as starting material.

The use of CaptEV reagent for cell culture supernatant has only been validated for use with samples sourced from cell culture supernatant. Other sample types have not been tested.

Protocol

The reagent is supplied in a 2X concentrated form. One (1) volume of reagent has to be mixed with one (1) volume of biofluid.

1. Harvest biofluid containing the extracellular vesicles of interest.
2. For cell culture supernatant, spin it at 3000g for 30 minutes at +4°C in order to eliminate floating cells and cells debris.
3. Filter biofluid gently through a 0.2 µm cut-off to eliminate large vesicles such as apoptotic bodies and most of the microvesicles.
4. Mix one volume of cleared, conditioned cell culture supernatant or serum and plasma with one volume of precipitation reagent.

The precipitation reaction can be easily scaled up or down depending on the needs of the final user. The precipitation reagent is more efficient than an ultrafiltration column when used in combination with immunocapture (Diagenode ExoIP™ kit). Thus, a protocol set up previously for concentrating cell culture supernatant can be used as is for precipitation before immunocapture.

5. Mix the precipitation mixture by gently inverting the reaction tube several times.
6. Incubate 1 hour at room temperature (RT) or at +4°C overnight (16 hours) under mild agitation.
7. Spin the precipitation mixture at 1500g for 30 minutes at +4°C.
8. Discard the supernatant.
9. Resuspend the pellet with the desired volume of PBS before downstream processing.

Typically, the pellet should be very easily resuspendable except if the cell culture supernatant contains poorly depleted serum.

Notice: If an exosome-rich pellet is processed by immunocapture (ExoIP™) afterwards, it is advised to keep the resuspension volume under 500 µl of PBS. Too large of a volume (→ 1 ml) is not beneficial, since bead-binding kinetics is dependent on the reaction volume.

10. Process the exosome-enriched suspension into immunocapture with the ExoIP™ kit or store it at -80°C if not used immediately.

At this stage, the exosome preparation is far from being pure. Further purification such as immunocapture (ExoIP™) of the target or gel filtration (e.g. Diagenode EVCleaner) need to be applied. Moreover, the exosome-rich preparation obtained by precipitation contains chemicals that enhance downstream immunocapture but may not be suitable for

direct processing by other techniques that require a very pure sample.

Related products

Product name	Cat. No.
C28030001	CaptEV cell culture
C28030002	CaptEV serum/plasma
C28020001	EVCleaner

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