iba Solutions For Life Sciences

Cell Selection & Expansion

Manual

CD81 FAB-TACS® EXOSOME ISOLATION KIT

human

for cell culture supernatant, serum and plasma

cat. no. 6-3281-004 (4 columns) and 6-3281-010 (10 columns)

1. GENERAL INFORMATION

Intended use: This kit is for research use only.

Components: Fab-TACS® Gravity Column filled with agarose matrix, 1 ml
6-6310-001 (Quantity: 4 / 10 columns)

CD81 Fab-Strep, human, lyophilized
6-8015-045 (Quantity: 4 / 10 vials)

100 mM Biotin stock solution for elution, 0,25 ml
6-6996-001 (Quantity: 1 / 2 vials)

Fab-TACS® Gravity Adapter
6-6331-001 (Quantity: 1 / 2 pcs)

Please note that buffer is not included.

Required reagents: Buffer with pH 7.4 (e.g. PBS, TBS or HEPES buffer depending on downstream application).

Optional: 0.2 µm cellulose acetate filter
0.22 µm polyethersulfone filter

Stability: 6 months after shipping.

Storage: Store all components at +2° C to +8° C.

Warnings: The agarose contains sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

2. TECHNICAL SPECIFICATIONS

- Columns are no "flow stop". **Avoid running the matrix dry!** Do not interrupt the procedure for more than 60 minutes.
- Capacity: $5x10^{10}$ exosomes.
- Reservoir volume: 10 ml.
- Fab-TACS® columns are designed for single use only.

3. PROTOCOL

3.1. Reagent preparation

Allow the reagents to equilibrate to room temperature (RT) prior to use. For a sterile isolation, work under a safety cabinet. **The following volumes will be sufficient for one selection process.**

3.1.1. Filtrate Buffer to remove interfering particles (recommended: 0.2 µm cellulose acetate filter).

3.1.2. Dissolve lyophilized Fab-Strep in 1 ml filtrated Buffer by carefully pipetting up and down. Do not vortex!

3.1.3. Prepare Biotin Elution Buffer (1 mM final concentration) by adding 40 µl 100 mM biotin solution to 4 ml Buffer. Mix thoroughly and filtrate (recommended: 0.2 µm cellulose acetate filter).

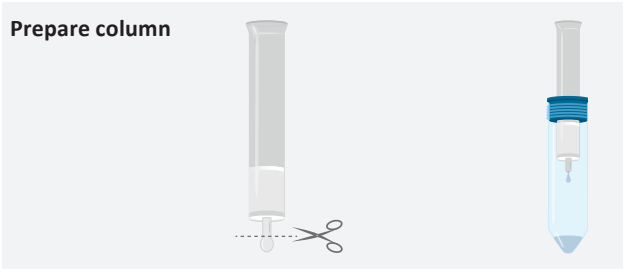
3.2. Sample preparation

3.2.1. Cell culture supernatants: Centrifuge cell culture supernatant at 3000 xg for 10 min or 2000 xg for 20 min **in advance**. Filtrate supernatant (recommended: 0.22 µm polyethersulfone filter. **Do not use cellulose acetate filters!**).

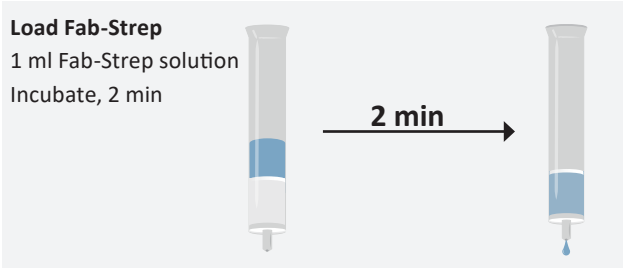
3.2.2. Serum and plasma: Centrifuge serum/plasma 2x at 3000 xg for 10 min. Filtrate supernatant (recommended: 0.22 µm polyethersulfone filter. **Do not use cellulose acetate filters!**).

3.3. Column preparation

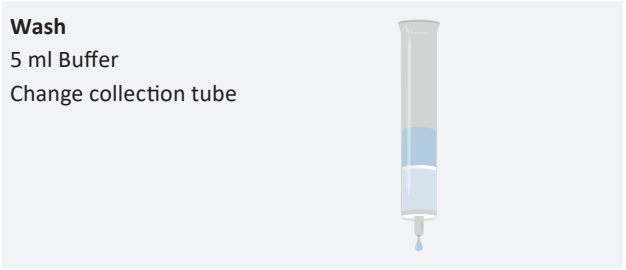
3.3.1. Remove the cap and cut the sealed end of the column at notch. Allow the storage solution to drain. Place the Fab-TACS® Gravity column into the Fab-TACS® Gravity Adapter. Add 5 ml buffer to remove sodium azide.



3.3.2. Apply the 1 ml Fab-Strep solution (3.1.2) onto the Fab-TACS® Gravity column. Let the Fab-Strep solution enter the packed bed completely. Incubate for 2 min.

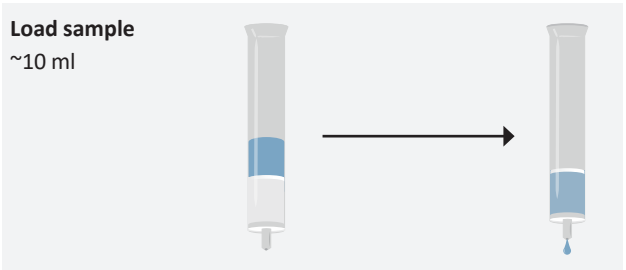


3.3.3. Wash the Fab-TACS® Gravity column with 5 ml Buffer. Discard effluent and change collection tube. The Fab-TACS® Gravity column is now ready for exosome isolation.

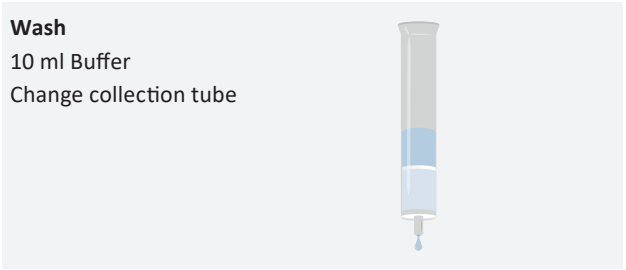


3.4. Exosome isolation with Fab-TACS® Gravity columns

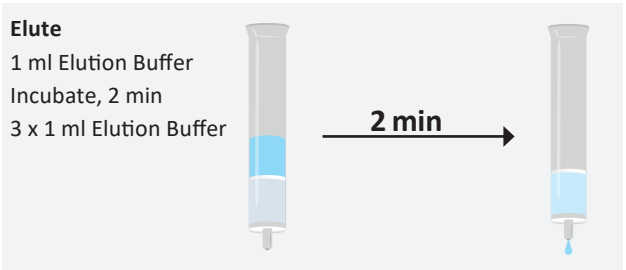
3.4.1. Loading – Apply sample (3.2) in steps of 1 ml (max. 20 ml). Collect flow-through containing unwanted material if needed.



3.4.2. Wash – Apply 10 ml Buffer. Let the buffer solution enter the gel bed completely. Collect flow through containing unwanted material.



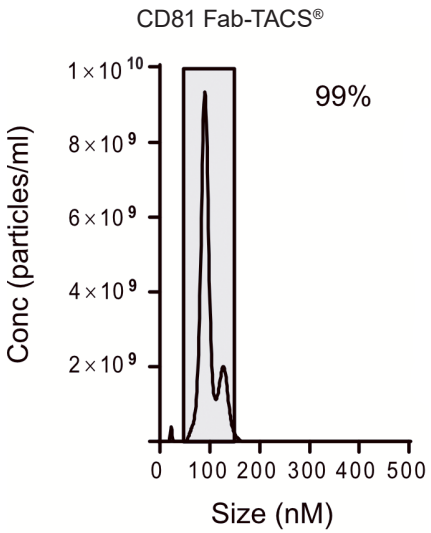
3.4.3. Elution – From this step on your effluent contains your target exosomes. Use a new collection tube. Apply 1 ml filtrated Biotin Elution Buffer (3.1.3), let the buffer enter the gel bed completely and incubate for 2 min. Elute target exosomes by applying 3 x 1 ml Biotin Elution Buffer.



3.4.4. Optional: Use size exclusion chromatography or hydrostatic filtration dialysis as an additional step to remove Biotin and Fabs for an ultra-pure exosome suspension.

4. EXAMPLE

Isolation of exosomes from HEK293 cell supernatant using the CD81 Fab-TACS® Exosome Isolation Kit. Extracellular vesicles were analyzed with the NanoSight LM10 instrument (Malvern Instruments) and data were processed using NTA software 2.3. 99% of particles ranged between 30 nm and 150 nm in diameter, indicating exosome enrichment.



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