Cell Selection & Expansion

! For research use only

Protocol

CD9 Fab-TACS® Exosome Agarose Column Starter Kit

Cat. no. 6-3319-002

human, for cell culture supernatant, serum and plasma

1. GENERAL INFORMATION & TECHNICAL SPECIFICATIONS

Kit components:

Cat. no.	Product	Quantity	Required/isolation
6-6310-300	Strep-Tactin® TACS Agarose Column, 0.3 ml	2	1
6-8019-150	CD9 Fab-Strep, human, lyophilized, 50 µg	1	20 µg
6-6996-001	Biotin stock solution, 100 mM, 250 µl	1	16 µl
6-3333-001	TACS Column Adapter (0.3 ml column)	1	1

Required: Buffer with pH 7.4 (e.g. PBS, TBS or HEPES buffer depending on downstream application), 0.2 µm cellulose acetate filter, 0.22 µm and 0.1 µm polyethersulfone filters.

Column specifications: Capacity: **2-3 x 10^9** targeted exosomes per column (sample-dependent)
Reservoir volume: 3 ml; **For single use only!**

Storage: Store all components at 2 - 8 °C. Store reconstituted Fab-Strep at -80 °C.

Stability: 6 months after shipping.

Shipping: Room temperature

Hazards: Products are not classified as hazardous according to (EC) No 1272/2008 [CLP]. Material Safety Data Sheets are provided.

2. INITIAL PREPARATIONS

2.1. 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**.

2.1.1. Filtrate approximately **15 ml** buffer to remove interfering particles (recommended: 0.2 µm cellulose acetate filter).

2.1.2. Dissolve lyophilized Fab-Strep in **1 ml** filtrated buffer by carefully pipetting up and down (avoid foam formation). **Do not vortex!**

Required per column: **20 µg** Fab-Strep in **400 µl** buffer. Store remaining solution in aliquots at **-80 °C** (stable for 6 months) if not required immediately. Avoid multiple freeze-thaw cycles.

2.1.3. Prepare 1 mM Biotin Elution Buffer by adding **16 µl** of the 100 mM Biotin stock solution to **1.6 ml** filtrated **buffer (2.1.1.)**. Mix thoroughly.

2.2. Sample preparation

2.2.1. Cell culture supernatants: Centrifuge cell culture supernatant at **3000 x g** for **10 min** or **2000 x g** for **30 min** in advance. Filtrate supernatant (recommended: 0.22 µm polyethersulfone filter. **Do not use cellulose acetate filters!).**

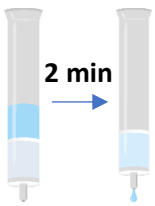
If cells need to be collected as well, first centrifuge supernatant at 300 x g for 10 min and continue with further centrifugation steps using the supernatant.

2.2.2. Serum and plasma: Sediment blood for **30 min** at **room temperature**. Centrifuge serum/plasma twice at **3000 x g** for **10 min**. Filtrate supernatant (recommended: 0.22 µm polyethersulfone filter. **Do not use cellulose acetate filters!**)

2.3. Column preparation

2.3.1. Remove the caps at the top and at the bottom of the column. Allow the storage solution (contains sodium azide) to drain. Place the Strep-Tactin® TACS Agarose Column into the TACS Column Adapter.

2.3.2. Wash the Strep-Tactin® TACS Agarose Column by applying **1 ml** buffer and allow the buffer solution to enter the packed bed completely.

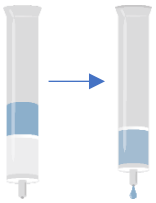


2.3.3. Load the **400 µl** Fab-Strep solution (2.1.2.) onto the Strep-Tactin® TACS Agarose Column. Let the Fab-Strep solution enter the packed bed completely. Incubate for **2 min**.

2.3.4. Wash the Strep-Tactin® TACS Agarose Column with **1 ml** buffer. Discard effluent and change collection tube. Strep-Tactin® TACS Agarose Column is now ready for exosome isolation.

Do not interrupt the procedure for more than 60 min.

3. PROTOCOL



3.1.1. Load

Apply prepared sample (2.2.) in steps of **1 ml** (max.: 7 ml in total). Collect flow-through containing unwanted material.

3.1.2. Wash

Apply **3 x 3 ml** buffer. (In each step: Let the buffer solution enter the gel bed completely). The agarose bed should now be white again.

3.1.3. Elute

From this step on your effluent contains your target exosomes. Use a **new collection tube**. Apply **400 µl** Biotin Elution Buffer (2.1.3.) and incubate for **5 min**. Elute exosomes by applying **3 x 400 µl** Biotin Elution Buffer.

3.1.4. Filter

Filter eluted exosome suspension through a 0.22 µm or 0.1 µm polyethersulfone filter. For yielding exosomes within a size range of 30 – 150 nm, we recommend using a 0.1 µm filter.

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

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If you have any questions, please contact

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We are here to help!

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