

Protocol

# Fab-TACS<sup>®</sup> affinity chromatographic exosome isolation

for serum, plasma or cell culture supernatants

## 1. REQUIRED REAGENTS & MATERIALS

Cat. no.	Product	Required/isolation
6-6310-001	Strep-Tactin <sup>®</sup> TACS Agarose Column, 1 ml	1
6-8015-150/6-8019-150	CD81 or CD9 Fab-Strep, 50 µg, lyophilized	50 µg
6-6325-001	Biotin stock solution, 100 mM, 1 ml	40 µl
6-6331-001	TACS Column Adapter (1 ml column)	1
	Buffer with pH 7.4 (e.g. PBS, TBS or HEPES buffer)	~25 ml
	0.2 µm cellulose acetate filter	1
	0.22 µm and 0.1 µm polyethersulfone filters	1 per pore size

## 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 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!**
- 2.1.3. Prepare 1 mM Biotin Elution Buffer by adding **40 µl** of the 100 mM Biotin stock solution to **4 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.

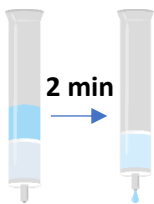
- 2.2.2.** Serum and plasma: Sediment blood for **30 min** at **room temperature**. Centrifuge serum/plasma twice at **3000 x g** for **10 min**. Filtrate supernatant (recommended: 0.22 µm polyethersulfone filter. **Do not use cellulose acetate filters!**)

## 2.3. Column preparation



**2.3.1. Remove** the cap and cut the sealed end of the column at notch. 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 **5 ml** buffer and allow the buffer solution to enter the packed bed completely.



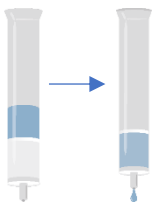
**2.3.3. Load** the **1 ml** Fab-Strep solution (2.1.2.) onto the Strep-Tactin® TACS Agarose Column. Let the Fab-Strep solution enter the packed bed completely. Incubate for **2 min**.

**2.3.4. Wash** the Strep-Tactin® TACS Agarose Column with **5 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.: 20 ml in total). Collect flow-through containing unwanted material.



To maximize the yield, the flow-through can be applied a second time.



### 3.1.2. Wash

Apply **10 ml** buffer. 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 **1 ml** Biotin Elution Buffer (2.1.3.) and incubate for **5 min**. Elute exosomes by applying **3 x 1 ml** 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!

