Cell Selection & Expansion

For research use only

Protocol

Fab-TACS® 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® 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

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!).**

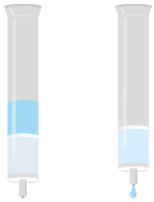
If cells need to be collected as well, first centrifuge supernatant at 300 x g for 10 min and continue with further centrifugation steps using the supernatant.

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.

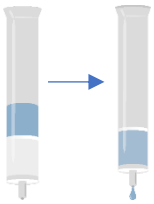


2.3.3. Load the **1 ml** Fab-Strep solution (2.1.2.) onto the Strep-Tactin® TACS Agarose Column. Let the Fab-Strep solution enter the packed bed completely. Incubate for **2 min**.

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 target cells by applying **3 x 1 ml** Biotin Elution Buffer.

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

Check our Downloads page

https://www.iba-lifesciences.com/resources/download-area/

for the latest version of this protocol

Info on warranty / licensing and trademarks available at:

www.iba-lifesciences.com/patents-licenses-trademarks/

If you have any questions, please contact

strep-tag@iba-lifesciences.com

We are here to help!

IBA Lifesciences GmbH · Rudolf-Wissell-Str. 28 · 37079 Goettingen · Germany · Phone + 49 551 50672-0 · info@iba-lifesciences.com