Cell Selection & Expansion

! For research use only

Protocol

CD81 Fab-TACS® affinity chromatographic cell isolation

human, for buffy coat

1. REQUIRED REAGENTS

Cat. no.	Product	Required/isolation
6-6310-001	Strep-Tactin® TACS Agarose Column, 1 ml	1
6-8015-150	CD81 Fab-Strep, human, lyophilized, 50 µg	50 µg
6-6325-001	Biotin stock solution, 100 mM, 1 ml	200 µl
6-6320-085	10x Buffer CI, 85 ml 10x PBS containing 10 mM EDTA and 5% BSA	~7-8 ml
6-6331-001	TACS Column Adapter (1 ml column)	1
	ddH_2O for Buffer CI dilution	

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. Prepare 1x Buffer CI from 10x stock by diluting with ddH_2O. Degas buffer before use, as air bubbles could block the column.

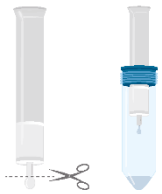
2.1.2. Dissolve **one vial** of lyophilized Fab-Strep (**50 µg**) in **1 ml** Buffer CI by carefully pipetting up and down (avoid foam formation). **Do not vortex!**

2.1.3. Prepare 1 mM Biotin Elution Buffer by adding **200 µl** of the 100 mM Biotin stock solution to **20 ml Buffer CI**. Mix thoroughly.

2.2. Sample preparation

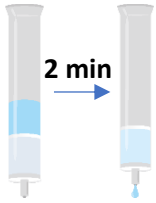
Dilute **5 ml** buffy coat in a 3:1 ratio with Buffer CI (dilute **5 ml** blood with **1.7 ml** Buffer CI). Mix gently by pipetting up and down. To remove clumps and to prevent aggregates, pass sample through a 40 µm nylon mesh before separation.

2.3. Column preparation



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



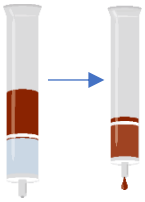
2.3.3. Load the **1 ml** Fab-Strep solution (2.1.2.) onto the Strep-Tactin® TACS Agarose Column. Let the Fab-Strep solution enter the packed bed completely. Incubate for **2 min**.

2.3.4. Wash the Strep-Tactin® TACS Agarose Column with **2 ml** Buffer CI. Discard effluent and change collection tube. The Strep-Tactin® TACS Agarose Column is now ready for cell isolation.

Do not interrupt the procedure for more than 60 min.

3. PROTOCOL

3.1. Cell isolation from buffy coat



3.1.1. Load
Apply diluted buffy coat (2.2.) in steps of **max. 5 ml**. Collect flow-through containing unlabeled cells.

3.1.2. Wash
Apply **4x 10 ml** Buffer CI. (In each step: Let the buffer solution enter the gel bed completely).

3.1.3. **Elute**
From this step on your effluent contains your target cells. Use a **new collection tube**. Apply **1 ml** Biotin Elution Buffer (2.1.3.) and incubate for **5 min**. Elute target cells by applying **9 ml** Biotin Elution Buffer. Elute a second time with additional **10 ml** Biotin Elution Buffer.

3.1.4. Optional: Apply additional **5 ml** of Buffer CI to the column and immediately centrifuge at **310 x g** for **2 min** to increase yield.

3.2. Further procedure

Centrifuge your eluted cell suspension for **10 min** at **300 x g**. Discard the supernatant and dissolve cell pellet in your desired buffer.

If you plan to continue with a biotin-sensitive assay, please remove biotin by washing with **50 ml** Buffer CI twice. Discard supernatant **completely.**

4. TROUBLESHOOTING

Low yield

Option 1:
Check for biotin contamination in your samples.

Option 2:
Use flow restrictor during sample loading.

Option 3:
Re-apply flow-through (depleted sample) to the column (3.1.1.).

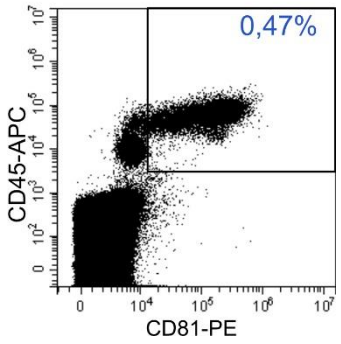
Low purity

Invert columns after each wash step three times.

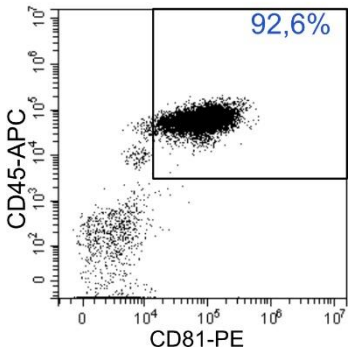
5. EXAMPLE DATA

Separation of CD81⁺ cells from buffy coat sample. Unlysed cells were stained with CD81-PE (5A6) and CD45-APC (2D1) and analyzed by flow cytometry (CytoFlex, BC). Dead cells were excluded from the analysis using PI staining. Doublet and debris discrimination were performed using different FSC/SSC signals.

Before isolation



After isolation



Watch this How-to video to see an exemplary isolation

https://www.youtube.com/watch?v=0PL_-uNjFZQ

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for the latest version of this protocol

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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