iba

Cell Selection & Expansion

! For research use only

Protocol

Cell isolation with Strep-Tactin® TACS Agarose columns (1 ml)

for whole blood, buffy coat, PBMCs or other single cell suspensions

1. REQUIRED REAGENTS

Cat. no.	Product	Required/isolation
6-6310-001	Strep-Tactin® TACS Agarose Column, 1 ml	1
	Protein of choice fused to a Twin-Strep-tag®	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	~ 63-72 ml

*Amount refers to a 50 kDa protein

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. Dilute your Twin-Strep-tag® fusion protein to a concentration of **50 µg/ml** with Buffer CI.

Titration of optimal isolation conditions might be necessary. The following instructions are an example for isolating cells with a **50 kDa** protein fused to a Twin-Strep-tag®.

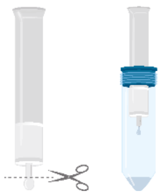
2.1.3. Prepare 1 mM Biotin Elution Buffer by adding **200 µl** of the 100 mM Biotin stock solution to **20 ml Buffer CI**. Mix thoroughly.

2.2. Sample preparation

2.2.1. For **PBMCs** or **other single cell suspensions**: Prepare cells and resuspend up to **3 x 10^8 cells/ 5 ml** Buffer CI.

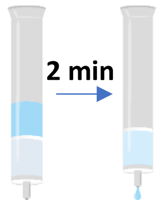
2.2.2. For **whole blood or buffy coat**: Dilute in a 3:1 ratio with Buffer CI, e.g. dilute **9 ml** whole blood with **3 ml** Buffer CI. Mix gently by pipetting up and down. To remove clumps and to prevent aggregates, pass sample through a 40 µm nylon mesh before separation.

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 1 ml Twin-Strep-tag® fusion protein (2.1.2.) onto the Strep-Tactin® TACS Agarose Column. Let the protein solution enter the packed bed completely. Incubate for **2 min**.

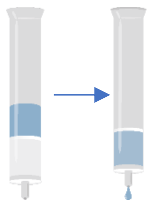
2.3.4. Wash the Strep-Tactin® TACS Agarose Column with **2 ml** Buffer CI. Discard effluent and change collection tube. The Strep-Tactin® TACS Agarose Column is now ready for cell isolation.

Do not interrupt the procedure for more than 60 min.

If you plan to isolate your cells from **PBMCs** or **other single cell suspensions** follow chapter **3.1**. For isolation from **whole blood or buffy coat** follow chapter **3.2**.

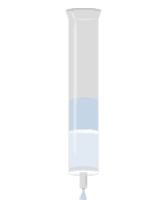
3. PROTOCOL

3.1. Cell isolation from PBMCs or other single cell suspensions

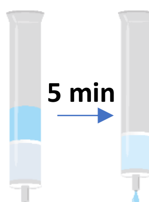


3.1.1. Load
Apply cell suspension (2.2.1.) in steps of **max. 5 ml**. Collect flow-through containing unlabeled cells.

If you expect more than 5 x 10^7 target cells you can apply the flow through a second time to maximize the yield.



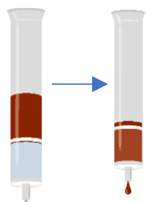
3.1.2. Wash
Apply **4x 10 ml** Buffer CI. (In each step: Let the buffer solution enter the gel bed completely).



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.

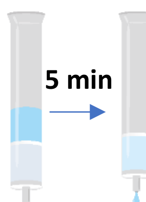
3.1.4. Optional: Apply additional **5 ml** of Buffer CI to the column and immediately centrifuge at **310 x g** for **2 min** to increase yield.

3.2. Cell isolation from whole blood or buffy coat



3.2.1. Load
Apply diluted whole blood or buffy coat (2.2.2.) in steps of **max. 5 ml**. Collect flow-through containing unlabeled cells.

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



3.2.3. 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.

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

3.3. 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:
Re-apply flow-through (depleted sample) to the column.

Low purity

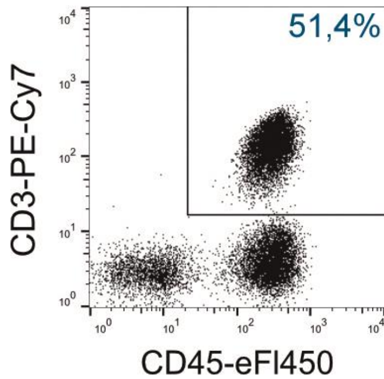
Invert columns after each wash step three times.

5. EXAMPLE DATA

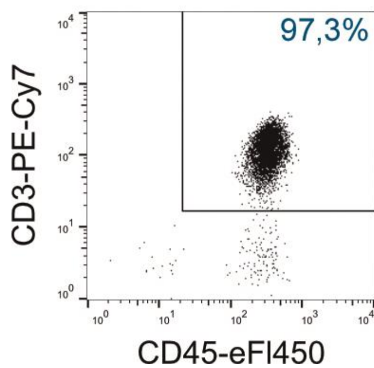
5.1. PBMCs

Separation of $CD3^+$ T cells from 5 ml PBMCs (containing 3 x 10^8 cells) using a Fab Fragment against CD3 fused to a Twin-Strep-tag®. Unlysed cells were stained with CD3-PE-Cy7 (OKT-3) / CD45-eFl450 (2D1) and analyzed by flow cytometry (CyAn ADP, BC). Dead cells were excluded from the analysis using PI staining. Doublet and debris discrimination were performed using different FSC/SSC signals.

PBMCs
Before isolation



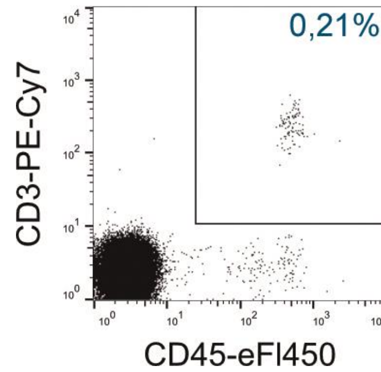
After isolation



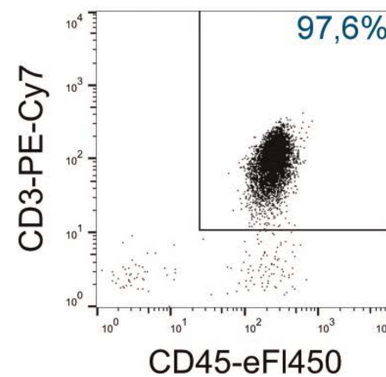
5.2. Buffy coat

Separation of $CD3^+$ T cells from buffy coat sample using a Fab Fragment against CD3 fused to a Twin-Strep-tag®. Unlysed cells were stained with CD3-PE-Cy7 (OKT-3) / CD45-eFl450 (2D1) and analyzed by flow cytometry (CyAn ADP, BC). Dead cells were excluded from the analysis using PI staining. Doublet and debris discrimination were performed using different FSC/SSC signals.

Buffy coat
Before isolation



After isolation



Watch this How-to video to see an exemplary isolation

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

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