Cell Selection & Expansion

! For research use only

Protocol

CD3 Fab-TACS® Agarose Column Starter Kit

Cat. no. 6-3201-002

human, for whole blood, buffy coat or PBMCs

1. GENERAL INFORMATION & TECHNICAL SPECIFICATIONS

Kit components:

Cat. no.	Product	Quantity	Required/isolation
6-6310-001	Strep-Tactin® TACS Agarose Column, 1 ml	2	1
6-8001-150	CD3 Fab-Strep, human, lyophilized, 50 µg	2	50 µg
6-6325-001	100 mM Biotin stock solution, 1 ml	1	200 µl
6-6320-025	10x Buffer CI, 25 ml 10x PBS containing 10 mM EDTA and 5% BSA	1	~7-8 ml
6-6331-001	TACS Column Adapter (1 ml column)	1	1

Required: ddH_2O for Buffer CI dilution

Column specifications: **1 x 10^8 target cells** out of
- 1 x 10^9 peripheral blood mononuclear cells (PBMCs)
- 5 - 20 ml (max. 50 ml) whole blood containing anticoagulant
- ~5 - 10 ml buffy coat

Reservoir volume: 10 ml; **For single use only!**

Storage: Store all components at 2 - 8 °C.
(Buffer CI may also be stored at 15 - 25 °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 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!**

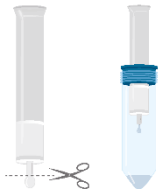
2.1.3. Prepare 1 mM Biotin Elution Buffer by adding **200 µl** of the 100 mM Biotin stock solution to **20 ml Buffer CI**. Mix thoroughly.

2.2. Sample preparation

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

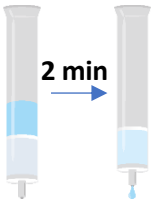
2.2.2. For **whole blood or buffy coat**: Dilute in a 3:1 ratio with Buffer CI, e.g. dilute **9 ml** whole blood with **3 ml** Buffer CI. Mix gently by pipetting up and down. To remove clumps and to prevent aggregates, pass sample through a 40 µm nylon mesh before separation.

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 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**.

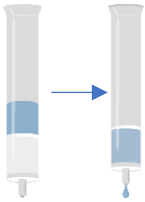
2.3.4. Wash the Strep-Tactin® TACS Agarose Column with **2 ml** Buffer CI. Discard effluent and change collection tube. The Strep-Tactin® TACS Agarose Column is now ready for cell isolation.

Do not interrupt the procedure for more than 60 min.

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

3. PROTOCOL

3.1. Cell isolation from PBMCs



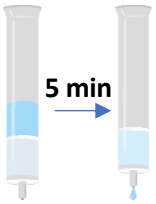
3.1.1. Load

Apply PBMCs (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).

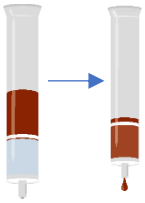


3.1.3. Elute

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:
Use flow restrictor during sample loading.

Option 3:
Re-apply flow-through (depleted sample) to the column (3.1.1./3.2.1.).

Low purity

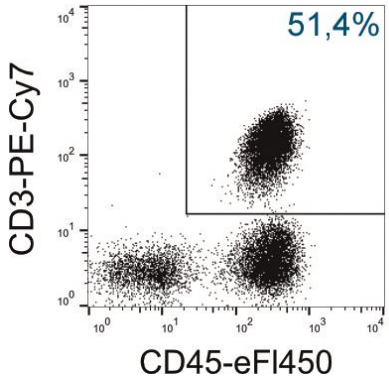
Invert columns after each wash step three times.

5. EXAMPLE DATA

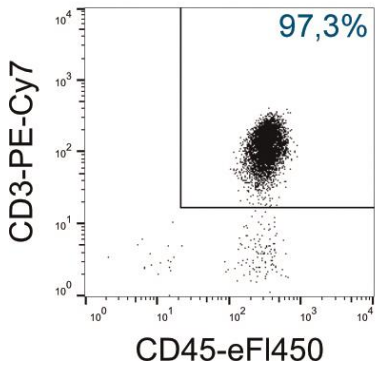
5.1. PBMCs

Separation of $CD3^+$ T cells from 5 ml PBMCs (containing 3 x 10^8 cells) using the CD3 Fab-TACS® Agarose Column Starter Kit. 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



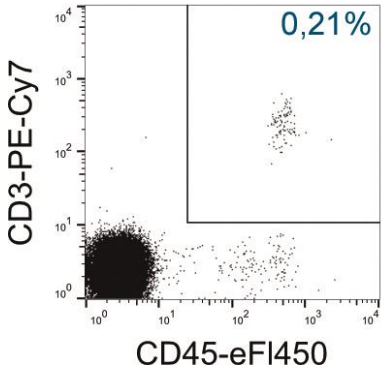
After isolation



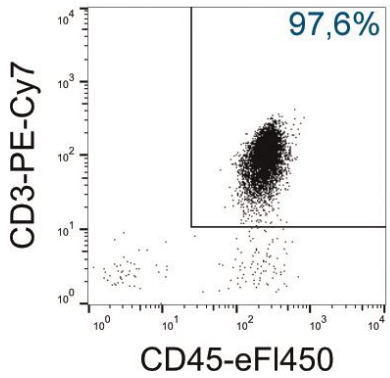
5.2. Buffy coat

Separation of $CD3^+$ T cells from buffy coat sample using the CD3 Fab-TACS® Agarose Column Starter Kit. 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



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for the latest version of this protocol and for troubleshooting

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If you have any questions, please contact

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We are here to help!

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