Cell Selection & Expansion

! For research use only

Protocol

CD31 Fab-TACS® Agarose Column Starter Kit

Cat. no. 6-3216-002

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

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
6-8016-150	CD31 Fab-Strep, human, lyophilized, 50 µg	2	50 µg
6-6325-001	Biotin stock solution, 100 mM, 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
6-6310-999	Flow Restrictor, pack of 5	1	1 piece

Required: ddH_2O for Buffer CI dilution

Column specifications: **4 x 10^7 target cells** out of
- 1 x 10^9 peripheral blood mononuclear cells (PBMCs) or other single cell suspensions
- 10 ml whole blood containing anticoagulant (citrate phosphate dextrose (CPD))
- 5 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.

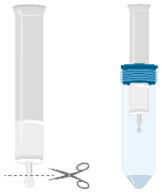
2.2. Sample preparation

2.2.1. For **PBMCs** and **other single cell suspensions**: Resuspend up to **3 x 10^8 cells/ 5 ml** Buffer CI. Cells are now ready for isolation.

2.2.2. For **buffy coat**: Dilute in a 1:1 ratio with Buffer CI, e.g. dilute **5 ml** whole blood with **5 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. **Whole blood** can be used undiluted. Continue with **2.2.3**.

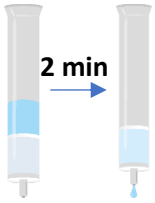
2.2.3. Perform the following three centrifugation steps at **room temperature** prior to cell isolation to reduce thrombocytes: **120 x g**, **10 min**; **300 x g**, **10 min**; **300 x g**, **10 min**. After each centrifugation step discard supernatant and refill with the same amount of Buffer CI.

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**.

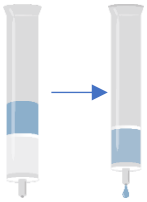
2.3.4. Wash the Strep-Tactin® TACS Agarose Column with **2 ml** Buffer CI. Discard effluent and change collection tube. The Strep-Tactin® TACS Agarose Column is now ready for cell isolation.

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 and other single cell suspensions



3.1.1. Load
Apply cells (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.

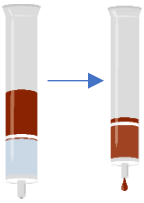
3.1.2. Wash
Apply **4x 10 ml** Buffer CI. (In each step: Let the buffer solution enter the gel bed completely).

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 (recommended for large cells): 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. Attach the flow restrictor to the column during blood loading.



3.2.2. Load
Apply whole blood or diluted buffy coat after thrombocyte elimination (2.2.3.) in steps of **max. 10 ml**. Remove flow restrictor after sample loading. 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

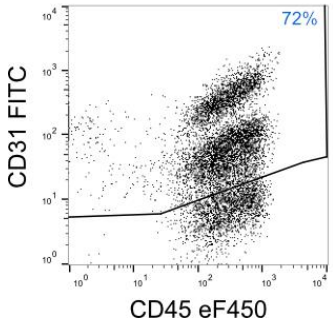
Invert columns after each wash step three times.

5. EXAMPLE DATA

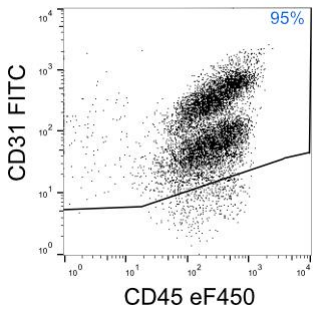
5.1. PBMCs

Separation of CD31⁺ cells from 5 ml PBMCs (containing 3x 10^8 cells) using the CD31 Fab-TACS® Agarose Column Starter Kit. Unlysed cells were stained with CD31-FITC (WM59) / CD45-eF450 (2D1) and analyzed by flow cytometry (CytoFlex, BC). Dead cells were excluded from the analysis using DAPI staining. Doublet and debris discrimination were performed using different FSC/SSC signals.

PBMCs
Before isolation



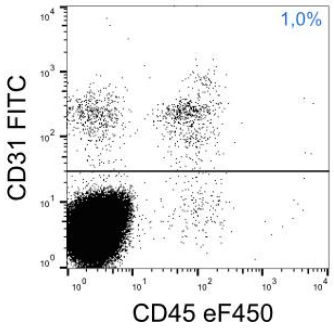
After isolation



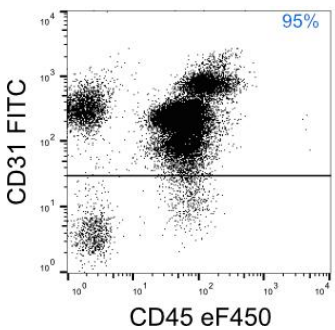
5.2. Buffy coat

Separation of CD31⁺ cells from buffy coat sample using the CD31 Fab-TACS® Agarose Column Starter Kit. Unlysed cells were stained with CD31-FITC (WM59) / CD45-eF450 (2D1) and analyzed by flow cytometry (CytoFlex, BC). Dead cells were excluded from the analysis using DAPI staining. Doublet and debris discrimination were performed using different FSC/SSC signals. Cells were pre-gated on living CD45⁺ leukocytes.

Buffy coat
Before isolation



After isolation



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If you have any questions, please contact

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We are here to help!

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