

**! For research use only**

Protocol

# CD3 Fab-TACS<sup>®</sup> 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 <sup>®</sup> TACS Agarose Column, 1 ml	2	1
6-8001-150	CD3 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

**Required:** ddH<sub>2</sub>O for Buffer CI dilution

**Column specifications:** **1 x 10<sup>8</sup> target cells** out of

- 1 x 10<sup>9</sup> 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

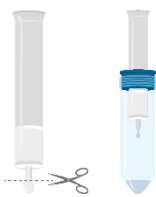
Allow the reagents to equilibrate to room temperature (RT) prior to use. For a sterile isolation, work under a safety cabinet. **The following volumes will be sufficient for one selection process.**

- 2.1.1. Prepare 1x Buffer CI from 10x stock by diluting with ddH<sub>2</sub>O. 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<sup>8</sup> 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.

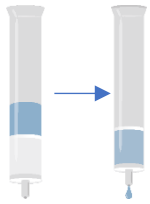


**Do not interrupt the procedure for more than 60 min.**

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  $5 \times 10^7$  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).

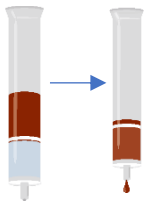


#### 3.1.3. Elute

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

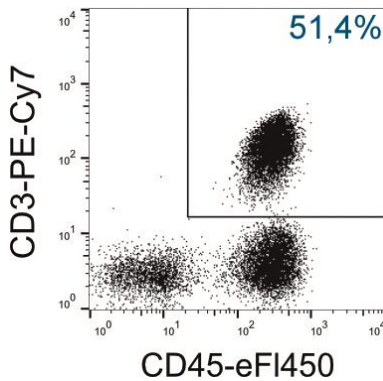
Invert columns after each wash step three times.

## 5. EXAMPLE DATA

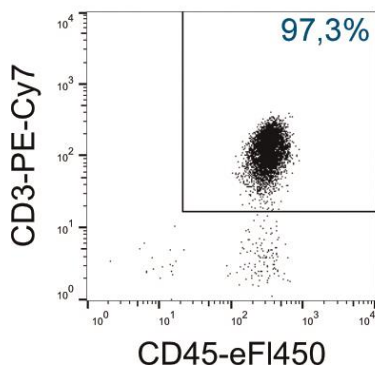
### 5.1. PBMCs

Separation of CD3<sup>+</sup> T cells from 5 ml PBMCs (containing  $3 \times 10^8$  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



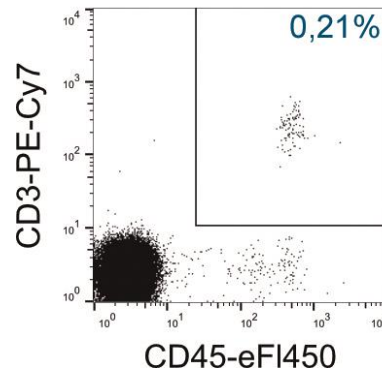
#### After isolation



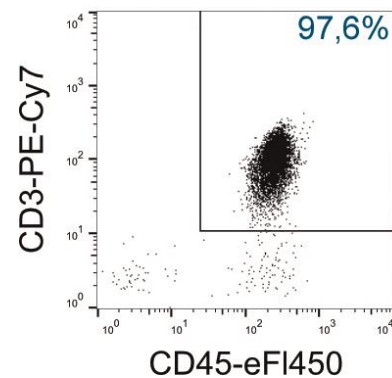
### 5.2. Buffy coat

Separation of CD3<sup>+</sup> 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





Watch this How-to video to see an exemplary isolation

[https://www.youtube.com/watch?v=0PL\\_-uNjFZQ](https://www.youtube.com/watch?v=0PL_-uNjFZQ)



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If you have any questions, please contact

[strep-tag@iba-lifesciences.com](mailto:strep-tag@iba-lifesciences.com)

We are here to help!

