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Protocol

# CD4/CD45RA Fab-TACS<sup>®</sup> Magnetic Microbead Starter Kit

Cat. no. 6-8000-121

human, for PBMCs

## 1. GENERAL INFORMATION & TECHNICAL SPECIFICATIONS

Kit components:

Cat. no.	Product	Quantity	Required/total cells		
			1 x 10 <sup>7</sup>	1 x 10 <sup>8</sup>	1 x 10 <sup>9</sup>
6-5510-005	Strep-Tactin <sup>®</sup> Magnetic Microbeads, 75 µl	2	15 µl	150 µl	1500 µl
6-8002-150	CD4 Fab-Strep, human, lyophilized, 50 µg	1	1 µg	10 µg	100 µg
6-8007-150	CD45RA Fab-Strep, human, lyophilized, 50 µg	1	1 µg	10 µg	100 µg
6-6325-001	Biotin stock solution, 100 mM, 1 ml	1	100 µl	150 µl	600 µl
6-6320-025	10x Buffer CI, 25 ml 10x PBS containing 10 mM EDTA and 5% BSA	1	3-4 ml	6-7 ml	18-19 ml

**Specifications:** For isolation **out of 1 x 10<sup>8</sup>** peripheral blood mononuclear cells (PBMCs)

**Required:** ddH<sub>2</sub>O for Buffer CI dilution; StrepMan Magnet (Cat. no. 6-5650-065)

**Storage:** Store all components at 2 - 8 °C. Store reconstituted Fab-Strep at -80 °C. (Buffer CI may also be stored at 15 - 25 °C)

**Stability:** 6 months after shipping.

**Shipping:** Room temperature

**Warnings:** 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

Volumes are suitable for isolating target cells out of **up to  $1 \times 10^7$**  PBMCs. For higher cell numbers, Fab-Strep and Strep-Tactin® Magnetic Microbead volumes should be upscaled linearly according to total cell numbers (e.g., for  $5 \times 10^7$  cells use 5x indicated Fab-Strep volume). Adapt other volumes according to **Table 1**.



**Cell labeling and isolation (3.1. and 3.2.) has to be performed at 4 °C.** Please make sure that all reagents and cells are accordingly refrigerated before starting the protocol. **The subsequent removal of reagents and washing (3.3 and 3.4) has to be performed at room temperature.**

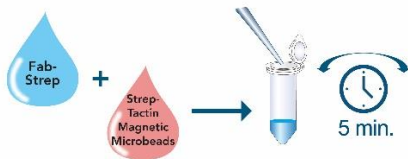
**2.1.1.** Prepare 1x Buffer CI by diluting stock with ddH<sub>2</sub>O.

**2.1.2. Optional:** Wash Strep-Tactin® Magnetic Microbeads before use to remove sodium azide. Add **1 ml** Buffer CI to required volume of microbeads (see 2.1.4.). Mix carefully and separate beads from buffer using a magnet. Discard supernatant and resuspend magnetic microbeads in Buffer CI (initial volume as in 2.1.4.).

**2.1.3.** Resuspend **50 µg** Fab-Strep in **1 ml** Buffer CI for a final concentration of **50 µg/ml**.



Store Fab-Strep solution in aliquots at **-80 °C** for up to **6 months**.



**2.1.4.** Mix **20 µl** (1 µg) Fab-Strep with **15 µl** Strep-Tactin® Magnetic Microbeads (vortex before pipetting!) in an Eppendorf tube. Incubate under constant gentle agitation for **5 min** (up to 24 h) at **4 °C**.

**2.1.5.** Prepare 1 mM Biotin Elution Buffer by diluting **100 µl** of 100 mM Biotin stock solution in **10 ml** Buffer CI. Mix thoroughly. Keep at **room temperature**.

### 2.2. Sample preparation

Prepare  **$1 \times 10^7$**  PBMCs in **30 µl** Buffer CI. Buffer CI volume should be upscaled linearly for higher cell numbers (e.g., use 5x 30 µl Buffer CI for  $5 \times 10^7$  total cells). Cells should be cooled down to **4 °C** before starting the protocol.

**Table 1: Recommended volumes & tube sizes for different cell numbers**

Starting cell number	Recommended tube size [ml]	Resuspension volume [ml]	Total Biotin Elution Buffer [ml]	3.3.1. [ml]
$\leq 1 \times 10^7$	15	5	10	5
$\leq 1 \times 10^8$	15	10	15	7.5
$\leq 1 \times 10^9$	50	30	60	30

## 3. PROTOCOL

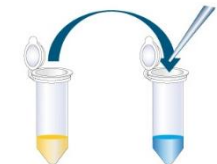
Steps for isolating CD4<sup>+</sup> CD45RA<sup>+</sup> naive T cells

1. CD4 positive cell isolation

2. CD45RA positive cell isolation

### 3.1. Cell labeling

Perform all steps at **4 °C**.



**3.1.1.** Add PBMCs to the pre-incubated Fab-Strep Microbead preparation (2.1.4.) and mix thoroughly by gentle pipetting.



**3.1.2.** Incubate for **10 min** under gentle constant agitation, e.g. on a roller mixer, to prevent cells from sedimentation. Continue with 3.2.1.

### 3.2. Magnetic cell isolation

Perform all steps at **4 °C**.



**3.2.1.** Add the incubated cells to **5 ml** Buffer CI. Mix thoroughly by gentle pipetting.



**3.2.2.** Incubate the tube on a magnet for **1 min**, remove entire supernatant carefully.



**3.2.3.** Resuspend cells in **5 ml** Buffer CI. Incubate the tube on a magnet for **1 min** (see 3.2.2.), remove entire supernatant carefully.



**3.2.4.** Repeat step 3.2.3. once. Continue with step 3.3.1.

### 3.3. Removal of magnetic microbeads

Perform all steps at **room temperature**.



**3.3.1.** Resuspend cells in **5 ml** Biotin Elution Buffer (2.1.5.). Mix by thoroughly by pipetting and incubate for **5 min** at **room temperature** on a roller mixer.



**3.3.2.** Incubate the tube on a magnet for **1 min**, collect entire supernatant carefully and transfer it to a new collection tube.



**3.3.3.** Repeat steps 3.3.1. and 3.3.2. once.

**3.3.4.** Pool the supernatants and collect cells by centrifugation (**400 x g, 6 - 10 min**).

Remove supernatant and resuspend cells in appropriate buffer or medium for further applications.



If further removal of magnetic microbeads is needed (e.g. for further positive enrichment steps), proceed to step **3.4**.

### 3.4. Removal of remaining magnetic microbeads

Perform all steps at **room temperature**.



**3.4.1.** Discard supernatant carefully. Resuspend cell pellet in **5 ml** Buffer CI and incubate for **5 min** under agitation (e.g. on a roller mixer) at **room temperature**.



**3.4.2.** Place tube back on the magnet (to remove any potential residual beads) and incubate for **3 min**.

**3.4.3.** After incubation, transfer supernatant to a **new tube** and centrifuge cells for **6 – 10 min** at **400 x g**.

**3.4.4.** Remove supernatant and resuspend cells in appropriate buffer or medium for further applications.



To perform further positive isolation or depletion steps, please start the protocol once more at **3.1**.

## 4. TROUBLESHOOTING

### Low yield

*Option 1:*

Titrate the ratio between Fab-Streps and Strep-Tactin® Magnetic Microbeads for different cell numbers.

*Option 2:*

Increase incubation time of cells with Fab-Strep-Microbead mix (3.1.2.).

*Option 3:*

Make sure that you carefully remove supernatants during incubation on the magnet (3.2.) without disrupting the binding of the microbeads to the magnet.

*Option 4:*

Check for biotin contamination in your samples.

### Low purity

Increase number of washing steps (3.2.)

### Microbead contamination

Make sure that you carefully remove supernatants during incubation on the magnet (3.3. and 3.4.) without disrupting the binding of the microbeads to the magnet.

### High amount of cell death

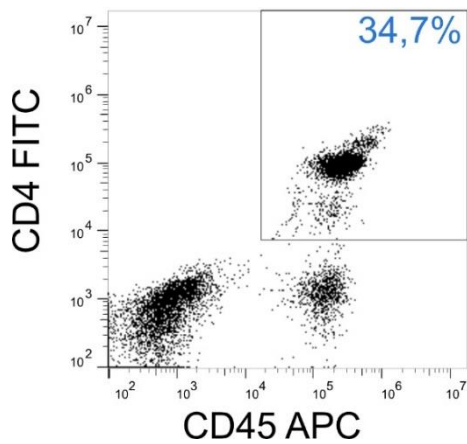
Make sure that you always work at the recommended temperatures.

## 5. EXAMPLE DATA

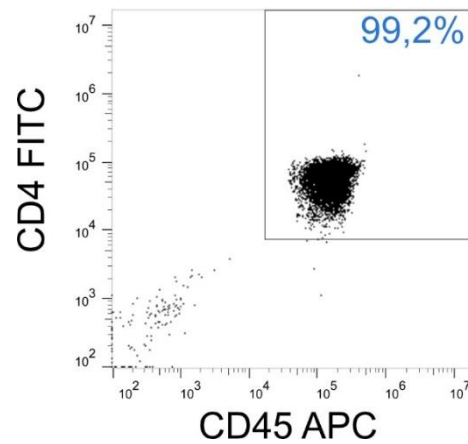
Isolation of CD4<sup>+</sup> T cells (1) and CD45RA<sup>+</sup> cells (2) from PBMCs. Unlysed cells were stained with CD4-FITC (SK3) / CD45-APC (2D1) or CD45RA-PE (HI100) / CD3-FITC (OKT-3) and analyzed by flow cytometry (CytoFlex, BC). Dead cells were excluded from the analysis using DAPI or PI staining. Doublet and debris discrimination were performed using different FSC/SSC signals.

### (1) CD4<sup>+</sup> T cell isolation

Before isolation

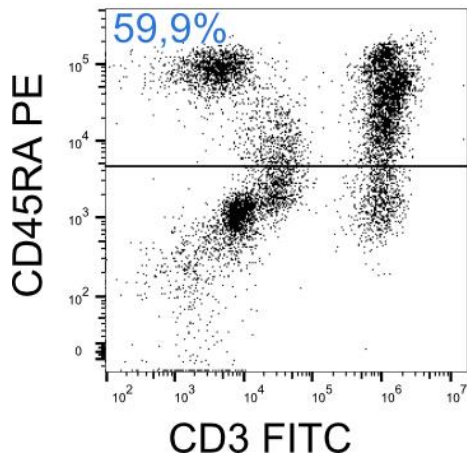


After isolation

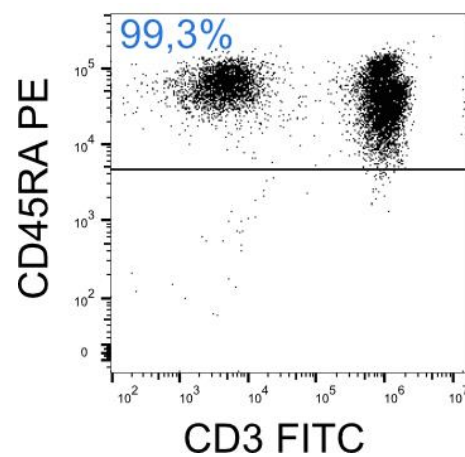


### (2) CD45RA<sup>+</sup> cell isolation

Before isolation



After isolation





Watch this How-to video to see an exemplary isolation

<https://www.youtube.com/watch?v=Er-HVYXluH8>



Check our Downloads page

<https://www.iba-lifesciences.com/resources/download-area/>

for the latest version of this protocol



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If you have any questions, please contact

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We are here to help!

