Cell Selection & Expansion

For research use only

Protocol

CD8/CD62L/CD45RA Fab-TACS® Magnetic Microbead Starter Kit

Cat. no. 6-8000-113

human, for PBMCs

1. GENERAL INFORMATION & TECHNICAL SPECIFICATIONS

Kit components:

Cat. no.	Product	Quantity	Required/total cells		
			1×10^7	1×10^8	1×10^9
6-5510-005	Strep-Tactin® Magnetic Microbeads, 75 µl	3	15 µl	150 µl	1500 µl
6-8003-150	CD8 Fab-Strep, human, lyophilized, 50 µg	1	1 µg	10 µg	100 µg
6-8005-150	CD62L 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	100 mM Biotin stock solution, 1 ml	1	100 µl	200 µl	600 µl
6-6320-085	10x Buffer CI, 85 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 1.5 x 10^8** peripheral blood mononuclear cells (PBMCs)

Required: ddH_2O 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: Blue ice

Warnings: Products are not classified as hazardous according to (EC) No 1272/2008 [CLP].
A 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 x 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 x 10^7 cells use 5x indicated Fab-Strep volume). Adapt other volumes according to **Table 1.**

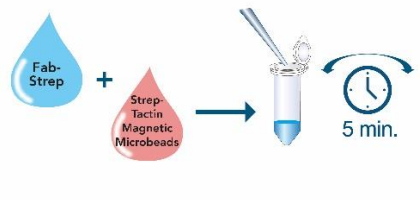
Cell labeling and isolation (3.1. and **3.2.) has to be performed at 4 °C.** Please make sure that all reagents and cells are accordingly refrigerated before starting the protocol. **The subsequent removal of reagents and washing (3.3** and **3.4) has to be performed at room temperature.**

2.1.1 Prepare 1x Buffer CI by diluting stock with ddH_2O.

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. 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 x 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 x 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**]
≤ 1 x 10^7	15	5	10	5
≤ 1 x 10^8	15	10	20	10
≤ 1 x 10^9	50	30	60	30

3. PROTOCOL

Steps for isolating $CD8^+CD62L^+CD45RA^-$ **central memory** T cells

1. CD8 positive cell isolation → 2. CD62L positive cell isolation → 3. CD45RA cell depletion

Steps for isolating $CD8^+CD62L^+CD45RA^+$ **naive** T cells

1. CD8 positive cell isolation → 2. CD62L positive cell isolation → 3. CD45RA positive cell isolation

3.1. Cell labeling

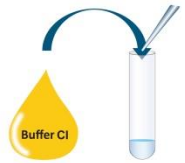
Perform all steps at **4 °C.**

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

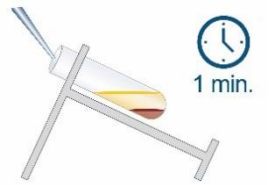
3.1.2. Incubate for **10 min** under gentle constant agitation, e.g. on a roller mixer, to prevent cells from sedimentation. Continue with 3.2.1.

3.2. Magnetic cell isolation

Perform all steps at **4 °C.**



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



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

For CD45RA depletion (3rd step for central memory T cell isolation) collect supernatant in step 3.2.2.. No further steps are required. The cells are ready for further downstream applications.

3.2.3. Repeat steps **3.2.1.** and **3.2.2** twice**.** 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 step **3.3.1.** and **3.3.2.** once.

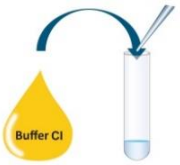
3.3.4. Pool the supernatants and collect cells by centrifugation **(400 x g, 6 - 10 min).**

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.** Make sure that biotin is removed entirely. Wash 3x with 10 ml Buffer CI and remove supernatants completely.

4. TROUBLESHOOTING

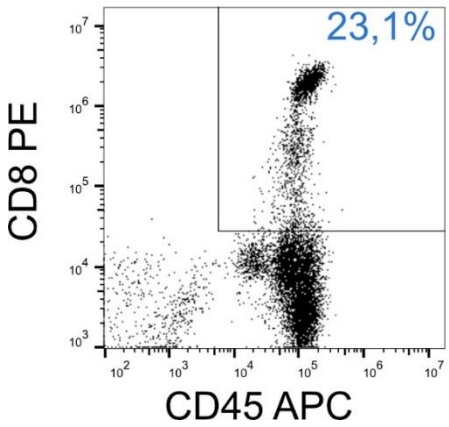
Low yield	*Option 1:* Titrate the ratio between Fab-Streps and Strep-Tactin® Magnetic Microbeads for different cell numbers. *Option 2:* Increase incubation time of cells with Fab-Strep-Microbead mix (3.1.2.). *Option 3:* Make sure that you carefully remove supernatants during incubation on the magnet (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

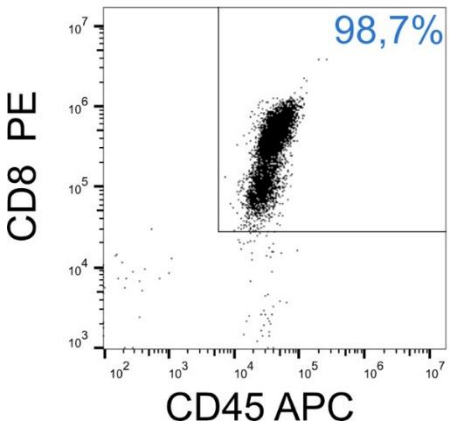
Examples of CD8$^+$ T cell isolation from PBMCs (1), CD62L$^+$ cells from T cell pre-enriched cells (2) and CD45RA$^+$ cells from PBMCs (3) as well as CD45RA depletion from PBMCs (4). Depending on experiment, unlysed cells were stained with CD8-PE (HIT8a), CD45-APC (2D1), CD8-PE-Cy7 (HIT8a), CD62L-PE (DREG-56), CD3-FITC (OKT-3), CD45RA-PE (HI100), CD45RA-APC (HI100) and/or CD45RO-FITC (UCHL1) and analyzed by flow cytometry (CytoFlex, BC or CyAn ADP, 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) CD8$^+$ T cell isolation

Before isolation

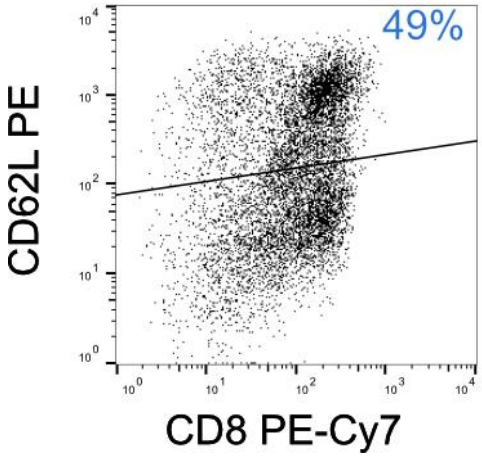


After isolation

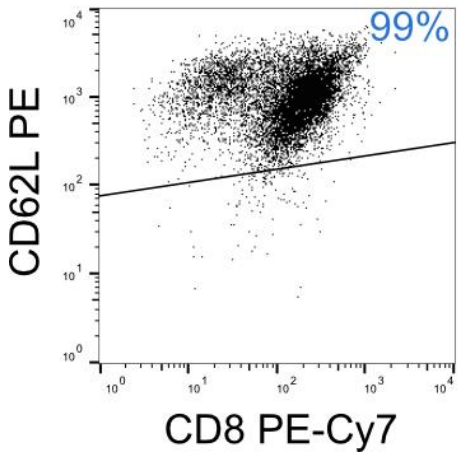


(2) CD62L$^+$ cell isolation

Before isolation

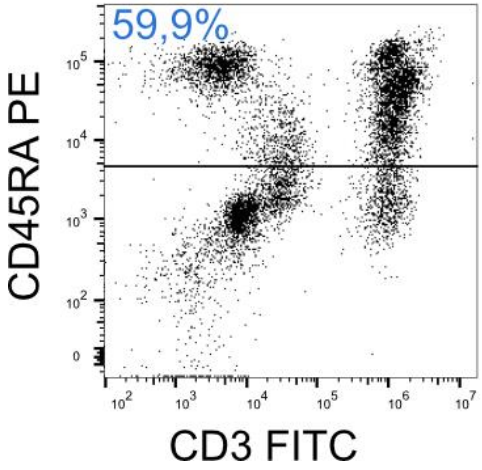


After isolation

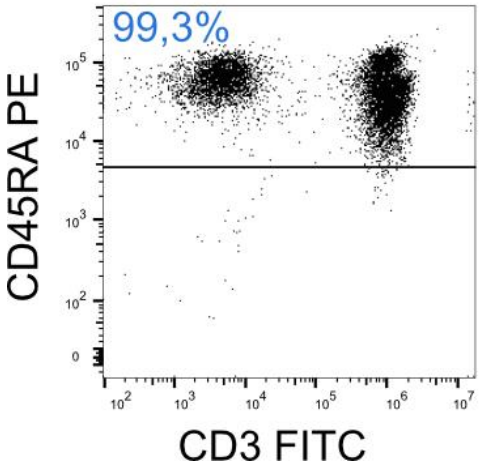


(3) CD45RA$^+$ cell isolation

Before isolation

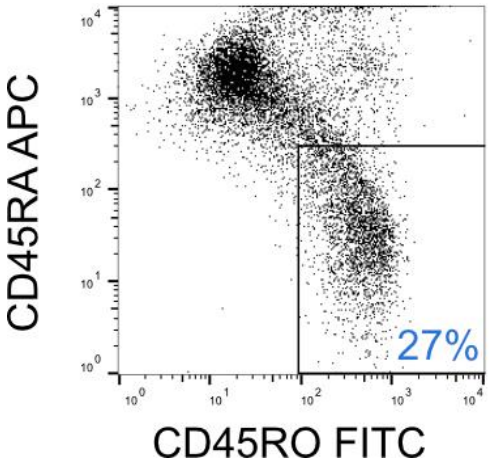


After isolation

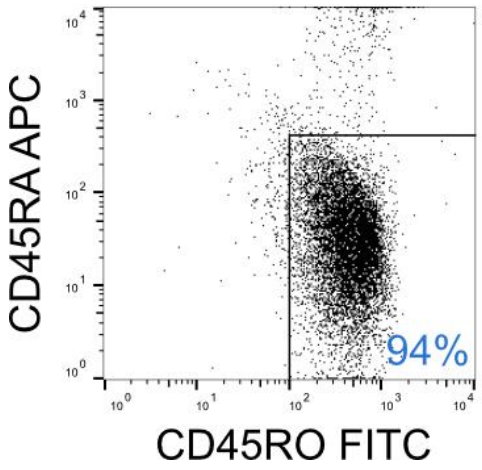


(4) CD45RA$^+$ cell depletion

Before depletion



After depletion



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If you have any questions, please contact

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We are here to help!