Cell Selection & Expansion

For research use only

Protocol

MHC I Streptamer® magnetic microbead cell isolation

for PBMCs

1. REAGENTS & MAGNET

Cat. no.	Product	Required/total cells	
		2 x 10^7	2 x 10^8
6-5510-050	Strep-Tactin® Magnetic Microbeads, 750 µl	30 µl	300 µl
6-7xxx-001	MHC I-Strep of choice, 40 µl*	8 µl*	80 µl*
6-6325-001	Biotin stock solution, 100 mM, 1 ml	100 µl	200 µl
6-6320-085	10x Buffer CI, 85 ml 10x PBS containing 10 mM EDTA and 5% BSA	3-4 ml	6-7 ml
6-5650-065	StrepMan Magnet		

*Based on standard concentration of 250 µg/ml. Check data sheet for individual differences in concentration.

Optional: Nylon filter tubes (e.g. Corning, Cat. no. 352235) to remove cell clumps

2. INITIAL PREPARATIONS

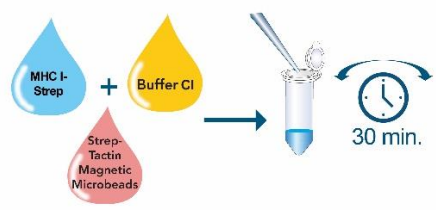
All steps have to be performed at **4 °C.** Please make sure that all reagents and cells are accordingly refrigerated before starting the protocol.

2.1. Reagent preparation

Volumes are suitable for isolating target cells out of **up to 2 x 10^7** PBMCs. For higher cell numbers, MHC I-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 2.5x indicated MHC I-Strep volume). Adapt other volumes according to **Table 1.**

2.1.1 Prepare 1x Buffer CI by diluting 10x 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 2.1.3.). Mix carefully and separate beads from buffer using a magnet. Discard supernatant and resuspend magnetic microbeads in Buffer CI (initial volume as in 2.1.3.).



2.1.3. Mix **8 µl** MHC I-Strep with **2 µl** Buffer CI and **30 µl** Strep-Tactin® Magnetic Microbeads. Incubate under constant gentle agitation for **30 min** (up to 24 h) at **4 °C** to generate MHC I Streptamers.

2.1.4. 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 **2 x 10^7** PBMCs in **210 µl** Buffer CI. Buffer CI volume should be upscaled linearly for higher cell numbers (e.g., use 2.5x 210 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 for different cell numbers

Starting cell number	Recommended tube size [**ml**]	Resuspension volume [**ml**]	Total Biotin Elution Buffer [**ml**]	3.3.1. [**ml**]
≤ 2 x 10^7	15	5	10	5
≤ 2 x 10^8	15	10	20	10

3. PROTOCOL

3.1. Cell labeling

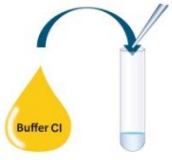
Perform all steps at **4 °C.**

3.1.1. Add the pre-incubated MHC I-Streptamers (2.1.3.) to the cells and mix thoroughly by gentle pipetting.

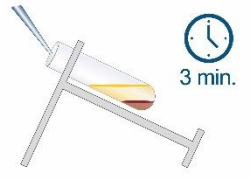
3.1.2. Incubate for **20 min** under gentle constant agitation, e.g. on a roller mixer, to prevent cells from sedimentation. Continue with 3.2.1.

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.

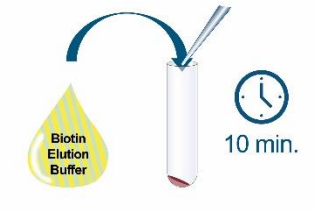


3.2.2. Incubate the tube on a magnet for **3 min**, remove entire supernatant carefully.

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 **4 °C.**



3.3.1 Resuspend cells in **5 ml** Biotin Elution Buffer (2.1.4.). Mix by thoroughly by pipetting and incubate for **10 min** at **4 °C** on a roller mixer.

3.3.2. Incubate the tube on a magnet for **3 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.

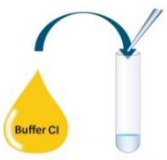
3.3.4. Pool the supernatants and collect cells by centrifugation **(400 x g, 6 - 10 min).**

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 **4 °C.**



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 at **4 °C**.

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.

4. TROUBLESHOOTING

Low yield	*Option 1:* Titrate the ratio between MHC I-Streps and Strep-Tactin® Magnetic Microbeads for different cell numbers. *Option 2:* Increase incubation time of cells with MHC I Streptamers (2.1.3.). *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 4 °C.

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If you have any questions, please contact

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We are here to help!

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