Cell Selection & Expansion

For research use only

Protocol

MHC I Streptamer® fluorescent cell staining

for flow cytometry analysis or sorting

1. REQUIRED REAGENTS

Cat. No.	Product	Required/2 x 10^6 total cells
6-5xxx-001	Strep-Tactin® or Strep-Tactin®**XT** PE or APC, 50 µl	1 µl
6-7xxx-001	MHC I-Strep of choice, 40 µl*	0.8 µl* / 0.2 µg
6-6325-001	Biotin stock solution, 100 mM, 1 ml	4 µl or 20 µl
6-6320-085	10x Buffer CI, 85 ml 10x PBS containing 10 mM EDTA and 5% BSA	~3 ml

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

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 staining **2 x 10^6** cells, e.g. peripheral blood mononuclear cells (PBMCs). Count your cell population before starting the experiment and adjust volumes accordingly.

2.1.1. Prepare 1x Buffer CI by diluting 10x stock with ddH_2O.

2.1.2. Incubate **0.8 µl** (0.2 µg) MHC I-Strep with **1 µl** fluorescent Strep-Tactin® or Strep-Tactin®**XT** in **10 µl** Buffer CI for at least **10 min** (up to 24 h) at **4 °C** to generate fluorescent MHC I Streptamers.

2.1.3. **Optional:** Prepare **1 mM or 5 mM** Biotin Elution Buffer by diluting **4 µl or 20 µl** of 100 mM Biotin stock solution in **400 µl** Buffer CI, respectively. Mix thoroughly. Keep at **4 °C**.

Please note: Removal of **Strep-Tactin®** conjugates requires **1 mM** Biotin Elution Buffer, whereas removal of **Strep-Tactin®XT** conjugates requires **5 mM** Biotin Elution Buffer.

3.2. Sample preparation

Cells should be cooled down to **4 °C** before starting the protocol.

2.2.1 If necessary, wash pre-cooled cell samples with **10 ml** Buffer CI to remove potentially interfering ingredients (e.g. biotin) by centrifuging at **400 x g** for **5 min.** Discard supernatant.

2.2.2. Resuspend cells in **20 µl** Buffer CI. Continue with the protocol (**3.1**).

For higher cell numbers, adjust cell concentration to **10^7 cells** per **100 µl** Buffer CI. Cell staining can be performed in 96-well, U- or V-bottom **microtiter plates** (up to 2 x 10^7 total cells) **or** V/round-bottom test **tubes** (> 2 x 10^7 total cells). Adjust wash steps accordingly.

3. PROTOCOL

3.1. Cell staining

Perform all steps at **4 °C.**

3.1.1. Add the pre-incubated fluorescent MHC I-Streptamers (**2.1.2.**) to the cells and mix thoroughly by gentle pipetting. **Optional**: Add additional staining antibodies if needed.

3.1.2. Incubate for **20 min** at **4 °C** in the dark.

3.1.3. Centrifuge sample at **400 x g** for **5 min** and discard supernatant.

3.1.4. Resuspend cells in **200 µl** (microtiter plate)/**2 ml** (tube) Buffer CI and wash by centrifuging at **400 x g** for **5 min** at **4 °C.** Discard supernatant.

3.1.5. Repeat step **3.1.4.** once**.**

Cells are ready for flow cytometric analysis or sorting. Propidium iodide or other live/dead discrimination is recommended. **For removal of staining reagents continue with 3.2.**

3.2. Removal of fluorescent MHC I Streptamers from cells

Perform all steps at **4 °C** after flow cytometric cell sorting**.**

Choose the correct biotin concentration: Strep-Tactin® conjugates: **1 mM** Biotin Elution Buffer; Strep-Tactin®**XT** conjugates: **5 mM** Biotin Elution Buffer.

3.2.1. Collect cells by centrifugation at **400 x g** for **5 min** and resuspend cell pellet in **200 µl** (up to 2 x 10^7 cells; microtiter plate)/ **1 ml** (> 2 x 10^7 cells; tube) Biotin Elution Buffer (2.1.3.). Incubate for **10 min** at **4 °C**.

3.2.2. Wash cells with **200 µl** (microtiter plate)/**2 ml** (tube) Buffer CI by centrifuging at **400 x g** for **5 min.** Discard supernatant.

3.2.3. **Repeat** incubation with Biotin Elution Buffer (see **3.2.1**.) and step **3.2.2**. once**.**

3.2.4. Resuspend cells in **200 µl** (microtiter plate)/**5 ml** (tube) Buffer CI and collect cells by centrifugation as in **3.2.2.** Discard supernatant.

3.2.5. **Repeat** step **3.2.4**. once

3.2.6. Resuspend cells in the appropriate buffer or medium for further applications.

4. TROUBLESHOOTING

Insufficient staining	Titrate optimal staining conditions: Keep the cell concentration of 10^7 cells/100 µl constant and increase the amount of MHC I Streptamers stepwise (2-, 3- and 4-fold). Example: Pre-incubate the following volumes for MHC I Streptamer® generation: 2-fold increase: 1.6 µl MHC I-Strep + 2 µl Strep-Tactin® PE in 20 µl Buffer CI, 3-fold increase: 2.4 µl MHC I-Strep + 3 µl Strep-Tactin® PE in 30 µl Buffer CI, 4-fold increase: 3.2 µl MHC I-Strep + 4 µl Strep-Tactin® PE in 40 µl Buffer CI.
No staining	Check for biotin contamination in your samples.

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If you have any questions, please contact

strep-tag@iba-lifesciences.com

We are here to help!

IBA Lifesciences GmbH · Rudolf-Wissell-Str. 28 · 37079 Goettingen · Germany · Phone + 49 551 50672-0 · info@iba-lifesciences.com