Cell Selection & Expansion

For research use only

Protocol

Fluorescent cell staining with (Twin-)Strep-tag® fusion proteins

For flow cytometry analysis or sorting

1. REQUIRED REAGENTS

Cat. No.	Product	Required/5 x 10^6 total cells
6-5xxx-001 or 2-156x-050	Strep-Tactin® or Strep-Tactin®**XT** fluorescent conjugate	75 ng
	Protein of choice fused to a (Twin-)Strep-tag®	200 ng of a 50 kDa protein
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

2. INITIAL PREPARATIONS

Cell staining 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) has to be performed at room temperature**

2.1. Reagent preparation

Volumes are suitable for **5 x 10^6** cells e.g., peripheral blood mononuclear cells (PBMCs) or other single cell suspensions. 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. Dilute your (Twin-)Strep-tag® fusion protein to a concentration of **50 – 250 µg/ml** with Buffer CI**.**

Titration of optimal staining conditions might be necessary. The following instructions are an example for staining cells with a **50 kDa** protein fused to a Twin-Strep-tag®. For weakly expressed or low affinity targets, we recommend using PE or APC conjugates for brighter staining.

2.1.3. Incubate **200 ng** of protein with **75 ng** fluorescent Strep-Tactin® or Strep-Tactin®**XT** conjugate (depending on the conjugate, a 1:10 pre-dilution might be necessary) for at least **10 min** (up to 24 h) at **4 °C**.

2.1.4. **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 **room temperature**.

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.

2.2. 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 **50 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 protein- fluorescent Strep-Tactin®/Strep-Tactin®**XT** preparation (**2.1.3.**) 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 Strep-Tactin®/Strep-Tactin®XT conjugates from cells

Perform all steps at **room temperature** 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 (2.1.4.). Incubate for **10 min** at **room temperature**.

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.

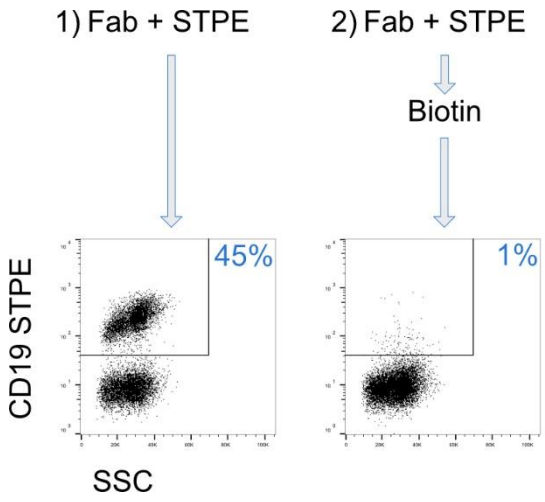
3.2.6. Resuspend cells in the appropriate buffer or medium for further applications.

4. TROUBLESHOOTING

Insufficient staining	Titrate optimal staining conditions: Keep the cell concentration of 10^7 cells/100 µl constant and increase the amount of protein-fluorescent Strep-Tactin® or Strep-Tactin® XT mix stepwise (2-, 3- and 4-fold). Example: Pre-incubate the following volumes: 2-fold increase (400 ng protein + 150 ng Strep-Tactin® or Strep-Tactin®XT conjugate).
Insufficient staining	Titrate optimal ratios between fluorescent conjugate and strep-tagged protein: Keep the amount of fluorescent conjugate constant and decrease or increase the amount of strep-tagged protein.
No staining	Check for biotin contamination in your samples.

5. EXAMPLE DATA

1) PBMCs were stained with pre-incubated CD19 Fab-Strep (Fab, a 50 kDa Fab fragment fused to a Twin-Strep-tag®) + Strep-Tactin® PE (STPE). 2) After staining, 1 mM biotin was added that caused the dissociation of STPE. Cells were 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.



Check our Downloads page

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

for the latest version of this protocol

Info on warranty / licensing and trademarks available at:

www.iba-lifesciences.com/patents-licenses-trademarks/

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