

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

for flow cytometry analysis or sorting

1. Required material

Cat. No.	Product	Required/5 x 10 ⁶ total cells
6-54xx-001 or 2-156x-050	Strep-Tactin®XT fluorescent conjugate	75 ng
-	Protein of choice fused to a Strep-tag®II or Twin-Strep-tag®	200 ng of a 50 kDa protein
6-6325-001	Biotin stock solution, 100 mM, 1 ml	20 µl
-	Buffer for cell isolation, e.g. 1x PBS containing 1 mM EDTA and 0.5% BSA	~ 30 ml

2. Initial preparations

2.1. Reagent preparation

Volumes are suitable for 5 x 10⁶ 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.



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** (3.2.) has to be performed at **room temperature**.

- 2.1.1.** Prepare a cell isolation buffer of choice, e.g. 1x PBS containing 1 mM EDTA and 0.5% BSA. The buffer should not contain biotin.
- 2.1.2.** Dilute your Strep-tag®II or Twin-Strep-tag® fusion protein to a concentration of **50 – 250 µg/ml** with cell isolation buffer.



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®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 **5 mM** Biotin Elution Buffer by diluting **20 µl** of 100 mM Biotin stock solution in **400 µl** cell isolation buffer. Mix thoroughly. Keep at room temperature.

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** cell isolation buffer 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** cell isolation buffer. Continue with the protocol (3.1).



For higher cell numbers, adjust cell concentration to **10⁷ cells per 100 µl** cell isolation buffer. Cell staining can be performed in 96-well, U- or V-bottom microtiter plates (up to 2 x 10⁷ total cells) or V/round-bottom test tubes (> 2 x 10⁷ 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®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) cell isolation buffer 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®XT conjugates from cells



Perform all steps at **room temperature** after flow cytometric cell sorting.

- 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 10⁷ cells; microtiter plate)/ **1 ml** (> 2 x 10⁷ 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) cell isolation buffer 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) cell isolation buffer 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

<i>Option 1</i>	Titrate optimal staining conditions: Keep the cell concentration of 10^7 cells/100 µl constant and increase the amount of protein-fluorescent 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®XT conjugate.
<i>Option 2</i>	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.