iba

Cell Selection & Expansion

For research use only

Protocol

Fab-TACS® fluorescent cell staining

For flow cytometry analysis or sorting

1. REQUIRED REAGENTS

Cat. No.	Product	Required/5 x 10^6 total cells
6-5000-001/6-5010-001	Strep-Tactin® PE or APC, 50 µl	1 µl
6-8xxx-150	Fab-Strep of choice, 50 µg, lyophilized	0.2 µg
6-6325-001	Biotin stock solution, 100 mM, 1 ml	4 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). 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. Resuspend **50 µg** Fab-Strep in **1 ml** Buffer CI for a final concentration of **50 µg/ml.**

Store reconstituted Fab-Strep in aliquots **at - 80 °C** for **up to 6 months**. Avoid multiple freeze-thaw cycles.

2.1.3. Incubate **4 µl** (0.2 µg**)** Fab-Strep with **1 µl** fluorescent Strep-Tactin® for at least **10 min** (up to 24 h) at **4 °C**.

2.1.4. **Optional:** Prepare **1 mM** Biotin Elution Buffer by diluting **4 µl** of 100 mM Biotin stock solution in **400 µl** Buffer CI. 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** 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 Fab-Strep- fluorescent Strep-Tactin® 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 fluorescent Strep-Tactin® and Fab-Streps 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 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

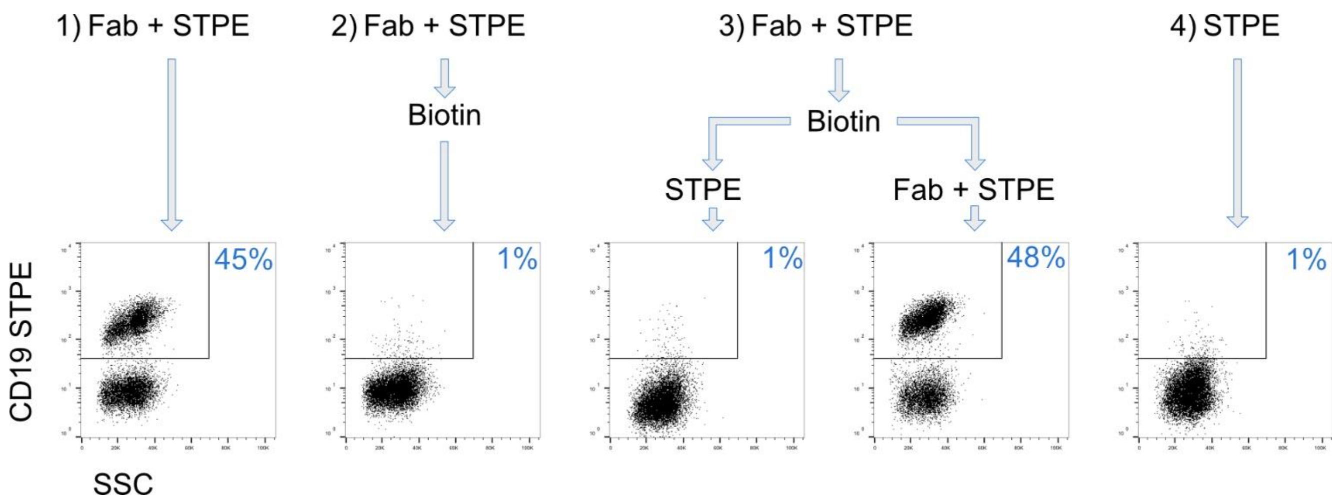
Titrate optimal staining conditions: Keep the cell concentration of 10^7 cells/100 µl constant and increase the amount of Fab-Strep-fluorescent Strep-Tactin® mix stepwise (2-, 3- and 4-fold). Pre-incubate the following volumes: 2-fold increase (8 µl Fab -Strep + 2 µl Strep-Tactin® PE/APC), 3-fold increase (12 µl Fab-Strep + 3 µl Strep-Tactin® PE/APC), 4-fold increase (16 µl Fab-Strep + 4 µl Strep-Tactin® PE/APC).

No staining

Check for biotin contamination in your samples.

5. EXAMPLE DATA

1) PBMCs were stained with pre-incubated CD19 Fab-Strep (Fab) + Strep-Tactin® PE (STPE). 2) After staining, 1 mM biotin was added that caused the dissociation of Fab + STPE. 3) After biotin addition and thorough washing, STPE was added to cells. No staining could be observed, indicating complete removal of CD19 Fab-Strep from the cell surface. Re-staining with Fab + STPE generated the same result as before biotin addition. 4) Negative control staining, indicating that STPE on its own does not cause a positive PE signal. 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.



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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