Cell Selection & Expansion

For research use only

Protocol

Cell isolation with Strep-Tactin® TACS Agarose columns (0.3 ml)

for splenocytes or other single cell suspensions

1. REQUIRED REAGENTS

Kit components:

Cat. no.	Product	Required/isolation
6-6310-300	Strep-Tactin® TACS Agarose Column, 0.3 ml	1
	Protein of choice fused to a Twin-Strep-tag®	20 µg*
6-6325-001	Biotin stock solution, 100 mM, 1 ml	60 µl
6-6320-085	10x Buffer CI, 85 ml 10x PBS containing 10 mM EDTA and 5% BSA	~2 ml
6-3333-001	TACS Column Adapter (0.3 ml column)	1

*Amount refers to a 50 kDa protein

2. INITIAL PREPARATIONS

Allow the reagents and the sample to equilibrate to room temperature (RT) prior to use. For a sterile isolation, work under a safety cabinet. **Reagent volumes will be sufficient for one selection process**.

2.1. Reagent preparation

2.1.1. Prepare 1x Buffer CI from 10x stock by diluting with ddH_2O. Degas buffer before use, as air bubbles could block the column.

2.1.2. Dilute your Twin-Strep-tag® fusion protein to a concentration of **50 µg/ml** with Buffer CI.

Titration of optimal isolation conditions might be necessary. The following instructions are an example for isolating cells with a **50 kDa** protein fused to a Twin-Strep-tag®.

2.1.3. Prepare 1 mM Biotin Elution Buffer by adding **60 µl** of the 100 mM Biotin stock solution to **6 ml** Buffer CI. Mix thoroughly.

2.2. Sample preparation

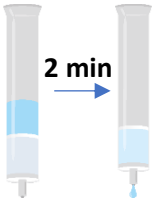
2.2.1. Prepare splenocytes in Buffer CI.

2.2.2. Adjust the concentration of the splenocytes to up to 5 x 10^7 total cells per ml. To remove clumps and to prevent aggregates, pass splenocytes through a 40 µm nylon mesh before isolation.

2.3. Column preparation

2.3.1. Remove the caps at the top and at the bottom of the column. Allow the storage solution to drain. Place the Strep-Tactin® TACS Agarose Column into the TACS Column Adapter.

2.3.2. Wash the Strep-Tactin® TACS Agarose Column by applying **1 ml** Buffer CI and allow the buffer solution to enter the packed bed completely.



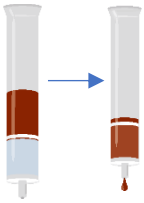
2.3.3. Load the **400 µl** Twin-Strep-tag® fusion protein (2.1.2.) onto the Strep-Tactin® TACS Agarose Column. Let the protein solution enter the packed bed completely. Incubate for **2 min**.

2.3.4. Wash the Strep-Tactin® TACS Agarose Column with **600 µl** Buffer CI. Discard effluent and change collection tube. Strep-Tactin® TACS Agarose Column is now ready for cell isolation.

Do not interrupt the procedure for more than 60 min.

3. PROTOCOL

3.1. Cell isolation from splenocytes



3.1.1. Load
Apply diluted splenocytes (2.2.2.) in steps of **max. 3 ml**. Collect flow-through containing unlabeled cells.

3.1.2. Wash
Apply **3x 3 ml** Buffer CI. (In each step: Let the buffer solution enter the gel bed completely). The agarose bed should now be white again.

3.1.3. Elute
From this step on your effluent contains your target cells. Use a **new collection tube**. Apply **400 µl** Biotin Elution Buffer (2.1.3.) and incubate for **5 min**. Elute target cells by applying **2.6 ml** Biotin Elution Buffer. Elute a second time with additional **3 ml** Biotin Elution Buffer.

3.3. Further procedure

Centrifuge your eluted cell suspension for **10 min** at **300 x g**. Discard the supernatant and dissolve cell pellet in your desired buffer.

If you plan to continue with a biotin-sensitive assay, please remove biotin by washing with **50 ml** Buffer CI twice. Discard supernatant **completely.**

4. TROUBLESHOOTING

Low yield

Option 1:
Check for biotin contamination in your samples.

Option 2:
Use flow restrictor during sample loading.

Option 3:
Re-apply flow-through (depleted sample) to the column (3.1.1./3.2.1.).

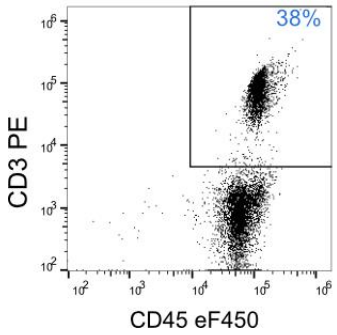
Low purity

Invert columns after each wash step three times.

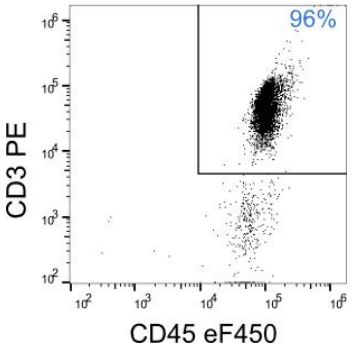
5. EXAMPLE DATA

Separation of $CD3^+$ T cells from mouse spleen using a Fab Fragment against CD3 fused to a Twin-Strep-tag®. Unlysed cells were stained with anti-mouse CD3-PE (145-2C11) / CD45-eF450 (1D4) and analyzed by flow cytometry (CytoFlex, BC). Dead cells were excluded from the analysis using DAPI staining. Doublet and debris discrimination were performed using FSC/SSC signals.

Before isolation



After isolation



Watch this How-to video to see an exemplary isolation

https://www.youtube.com/watch?v=bAaMgNlkTDI

Check our Downloads page

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

for the latest version of this protocol

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If you have any questions, please contact

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We are here to help!

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