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

for splenocytes or other single cell suspensions

1. Required material

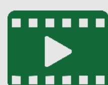
Cat. No.	Product	Required/isolation
6-6310-300	Strep-Tactin® TACS Agarose Column, 0.3 ml	1
-	Protein of choice fused to a Strep-tag®II or Twin-Strep-tag®	20 µg*
6-6325-001	Biotin stock solution, 100 mM, 1 ml	60 µl
-	Buffer for cell isolation, e.g. 1x PBS containing 1 mM EDTA and 0.5% BSA	~ 20 ml
6-3333-001	TACS Column Adapter (1 ml column)	1

*Amounts refer to a 50 kDa Twin-Strep-tag® protein

2. Initial preparations

2.1. Reagent preparation

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



Watch this How-to video to see an exemplary isolation:
https://www.youtube.com/watch?v=bAaMgNIkTDI

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. Degas buffer before use, as air bubbles could block the column.

2.1.2. Dilute your Strep-tag®II or Twin-Strep-tag® fusion protein to a concentration of **50 µg/ml** with cell isolation buffer.

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 diluting **60 µl** of 100 mM Biotin stock solution in **6 ml** cell isolation buffer. Mix thoroughly.

2.2. Sample preparation

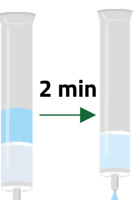
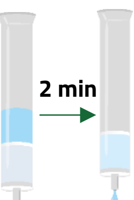
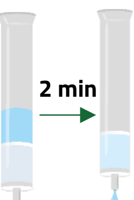
2.2.1. Prepare splenocytes in cell isolation buffer.

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 cap and cut the sealed end of the column at notch. 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** cell isolation buffer and allow the buffer solution to enter the packed bed completely.

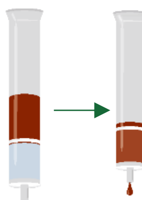
2.3.3. Load 400 µl Twin-Strep-tag® fusion protein (2.1.2.) onto the Strep-Tactin® TACS Agarose Column. Let the protein solution enter the packed bed completely. Incubate for **2 min**.

2.3.4. Wash the Strep-Tactin® TACS Agarose Column with **600 µl** cell isolation buffer. Discard flow-through and change collection tube. The 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** cell isolation buffer. (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 flow-through 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.

3.2. 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** cell isolation buffer twice. Discard supernatant completely.

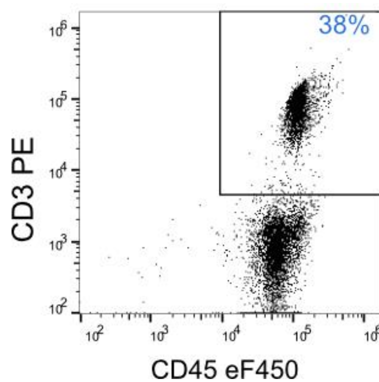
4. Troubleshooting

Low yield	
Option 1	Check for biotin contamination in your samples.
Option 2	Re-apply flow-through (depleted sample) to the column.
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