iba

Cell Selection & Expansion

! For research use only

Protocol

CD8 Fab-TACS® Agarose Column Starter Kit

Cat. no. 6-3305-002

mouse, for splenocytes

1. GENERAL INFORMATION & TECHNICAL SPECIFICATIONS

Kit components:

Cat. no.	Product	Quantity	Required/isolation
6-6310-300	Strep-Tactin® TACS Agarose Column, 0.3 ml	2	1
6-8505-150	CD8 Fab-Strep, mouse, lyophilized, 50 µg	1	20 µg
6-6996-001	100 mM Biotin stock solution, 250 µl	1	60 µl
6-6320-025	10x Buffer CI, 25 ml 10x PBS containing 10 mM EDTA and 5% BSA	1	~2 ml
6-3333-001	TACS Column Adapter (0.3 ml column)	1	1

Required: ddH_2O for Buffer CI dilution

Column specifications: **<u>2 x 10^7 target cells</u>** out of 1 x 10^8 splenocytes
Reservoir volume: 3 ml; **For single use only!**

Storage: Store all components at 2 - 8 °C. Store reconstituted Fab-Strep at -80 °C.
(Buffer CI may also be stored at 15 - 25 °C)

Stability: 6 months after shipping.

Shipping: Room temperature

Hazards: Products are not classified as hazardous according to (EC) No 1272/2008 [CLP].
Material Safety Data Sheets are provided.

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**.

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. Dissolve **one vial** of lyophilized Fab-Strep (**50 µg**) in **1 ml** Buffer CI by carefully pipetting up and down (avoid foam formation). **Do not vortex!**

Required per column: **20 µg** Fab-Strep in **400 µl** Buffer CI. Store remaining Fab-Strep solution at -80 °C (stable for 6 months) if not required immediately

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 1 – 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.

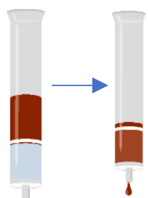
2.3.3. Load the **400 µl** Fab-Strep solution (2.1.2.) onto the Strep-Tactin® TACS Agarose Column. Let the Fab-Strep solution enter the packed bed completely. Incubate for **2 min**.

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.

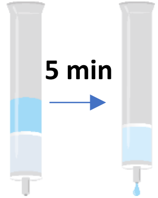
3. PROTOCOL

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.

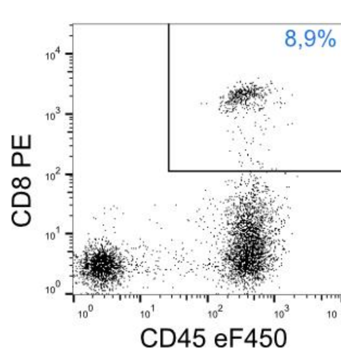
Option 3:
Re-apply flow-through (depleted sample) to the column (3.1.1./3.2.1.).

Low purity

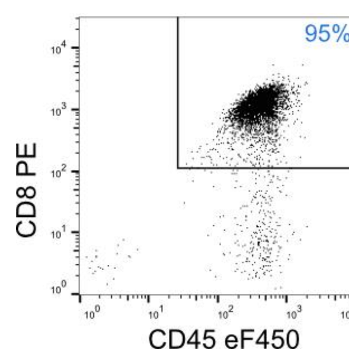
Invert columns after each wash step three times.

5. EXAMPLE DATA

Separation of CD8⁺ T cells from mouse spleen using the CD8 Fab-TACS® Agarose Column Starter Kit. Unlysed cells were stained with anti-mouse CD8-PE (53-6.7) / 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

Check our Downloads page

www.iba-lifesciences.com/download-area.html

for the latest version of this protocol

Info on warranty / licensing and trademarks available at:

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If you have any questions, please contact

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We are here to help!

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