iba Solutions For Life Sciences

Cell Selection & Expansion

Manual

CD4 FAB-TACS® GRAVITY KIT

mouse, cat. no. 6-6401-004 (4 columns) and 6-6401-010 (10 columns)

1. GENERAL INFORMATION

Intended use: This kit is for research use only.

Components: Fab-TACS® Gravity Column filled with cell-grade agarose matrix, 1 ml
6-6310-001 (Quantity: 4 / 10 columns)

CD4 Fab-Strep, mouse, lyophilized
6-8501-045 (Quantity: 4 / 10 vials)

100 mM Biotin stock solution for elution of cells, 1 ml
6-6325-001 (Quantity: 1 / 2 vials)

Buffer CI (10x) for cell isolation, 85 ml
6-6320-085 (Quantity: 1 / 1 bottle)

Storage: Store all components at +2° C to +8° C.
(Buffer CI may also be stored at 15 – 25° C)

Optional: Fab-TACS® Gravity Adapter: 6-6331-001

Warnings: The cell-grade agarose contains sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

2. TECHNICAL SPECIFICATIONS

- Columns are no "flow stop". **Avoid running the matrix dry!**
- Capacity per column: $1x10^8$ target cells.
- Reservoir volume: 10 ml.
- Fab-TACS® columns are designed for single use only.
- Buffer CI (1x): PBS contaning 1 mM EDTA and 0.5% BSA.

3. PROTOCOL

3.1. 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.**

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

3.1.2. Dissolve lyophilized Fab-Strep (1 vial) in 1 ml Buffer CI by carefully pipetting up and down. Do not vortex!

3.1.3. Adjust the concentration of the splenocytes to a number of $1–5x10^7$ total cells per mL. To remove clumps and to prevent aggregates, pass splenocytes through a 40 µm nylon mesh before isolation.

3.1.4. Prepare Biotin Elution Buffer by adding 200 µl 100 mM biotin solution to 20 ml Buffer CI. Mix thoroughly.

3.2. Column preparation

3.2.1. Remove the cap and cut the sealed end of the column at notch. Allow the storage solution to drain. Place the Fab-TACS® Gravity column into the Fab-TACS® Gravity Adapter.

3.2.2. Wash the Fab-TACS® Gravity column by applying 5 mL Buffer CI and allow the buffer solution to enter the packed bed completely.

Prepare column

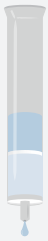
Wash
5 ml Buffer CI

3.2.3. Apply the 1 ml Fab-Strep solution (3.1.2) onto the Fab-TACS® Gravity column. Let the Fab-Strep solution enter the packed bed completely. Incubate for 2 min.

Load Fab-Strep
1 ml Fab-Strep solution
Incubate, 2 min

3.2.4. Wash the Fab-TACS® Gravity column with 2 ml Buffer CI. Discard effluent and change collection tube. The Fab-TACS® Gravity column is now ready for cell isolation.

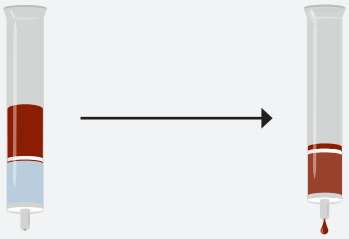
Wash
2 ml Buffer CI
Change collection tube



3.3. Cell isolation with Fab-TACS® Gravity columns

3.3.1. Loading – Apply diluted splenocytes (3.1.3) in steps of max. 10 ml. Collect flow-through containing the negative fraction.

Load cell suspension
max. 10 ml per step



3.3.2. Wash – Apply 3 times 10 ml Buffer CI. In each step: let the buffer solution enter the gel bed completely. Collect flow through containing unlabeled cells and combine with the effluent from step 3.3.1. The agarose bed should now be white again.

Wash
3x 10 ml Buffer CI
Change collection tube

3.3.3. Elution – From this step on your effluent contains your target cells. Use a new collection tube. Apply 1 ml Biotin Elution Buffer (3.1.4) and incubate for 5 min. Elute target cells by applying 9 ml Biotin Elution Buffer.

3.3.4. Elute with additional 10 ml Biotin Elution Buffer.

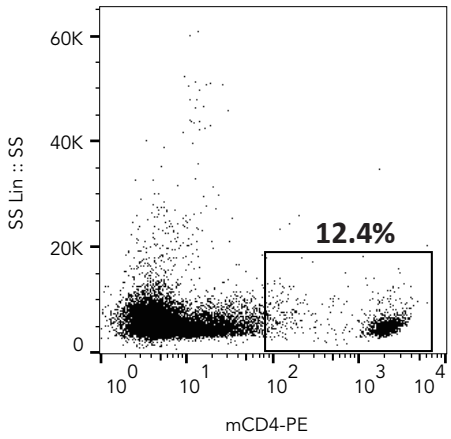
Elute
1 ml Elution Buffer
Incubate, 5 min
9 ml Elution Buffer

10 ml Elution Buffer

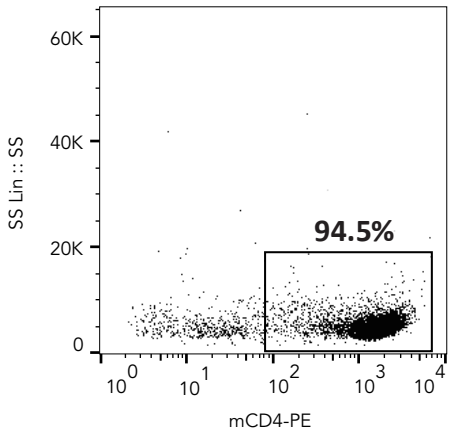
4. EXAMPLE ANALYSIS

Separation of CD4⁺ T cells from mouse spleen using the CD4 Fab-TACS® Gravity Kit, mouse. Unlysed cells were stained with anti-mouse CD4-PE (GK 1.5) and analyzed by flow cytometry (CytoFlex, BC). Dead cells were excluded from the analysis using DAPI staining. Doublet and debris discrimination were performed using different FSC/SSC signals.

Before separation



After separation



IBA GmbH · Rudolf-Wissell-Str. 28 · 37079 Goettingen · Germany · Phone +49 551 50672-0 · Fax +49 551 50672-181 · info@iba-lifesciences.com

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