iba Solutions For Life Sciences

Cell Selection & Expansion

Manual

CD3 FAB-TACS GRAVITY ISOLATION KIT

human, whole blood, cat. no. 6-6201-004 (4 columns) and 6-6201-010 (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)

CD3 Fab-Strep, human, lyophilized
6-8001-191 (Quantity: 1 / 2 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)

Fab-TACS Flow Restrictor, pack of 5

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: 15 ml whole blood containing anticoagulant. We recommend citrate phosphate dextrose (CPD).
- Reservoir volume: 10 ml.
- Fab-TACS columns are designed for single use only.
- Buffer CI: PBS contaning 1 mM EDTA and 0.5% BSA.

3. PROTOCOL

3.1. Reagent preparation

Allow the reagents, except the Fab-TACS Gravity column, to equilibrate to room temperature (RT) prior to use. Do not equilibrate the Fab-TACS Gravity column at RT in order to prevent the formation of air bubbles in the matrix. 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.

3.1.2. Dissolve lyophilized Fab in 1 ml Buffer CI by carefully pipetting up and down. Do not vortex! Transfer 200 µl Fab solution **each** into a fresh reagent tube. **The 200 µl Fab aliquots may be stored at -20° C.**

3.1.3. Add 800 µl Buffer CI to 200 µl Fab solution. The Fab solution is now ready for usage.

3.1.4. Dilute whole blood in a 1:3 ratio with Buffer CI, e.g. dilute 9 ml whole blood with 3 ml Buffer CI. Mix gently by pipetting up and down. To remove clumps and to prevent aggregates, pass whole blood through a 30 µm nylon mesh before separation.

3.1.5. 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 8 mL Buffer CI and allow the buffer solution to enter the packed bed completely.

Prepare column

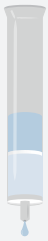
Wash
8 ml Buffer CI

3.2.3. Apply the 1 ml Fab solution (3.1.3) onto the Fab-TACS Gravity column. Let the Fab solution enter the packed bed completely. Incubate for 2 min.

Load Fab
1 ml Fab 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

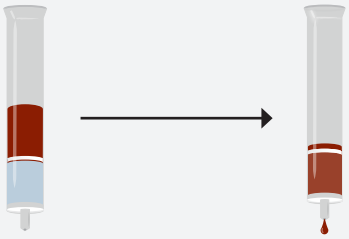


3.3. Cell isolation with Fab-TACS Gravity columns

3.3.1. Loading – Apply whole blood (3.1.4) in steps of max. 10 ml. Collect flow-through containing unlabeled cells.

Note: To maximize yield you may attach the flow restrictor during the blood loading.

Load whole blood
max. 10 ml per step



3.3.2. Wash – Apply 4 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.

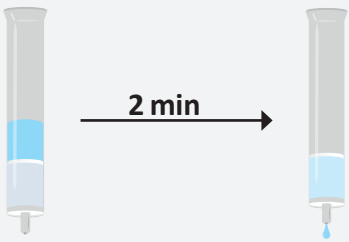
Wash
4x 10 ml Buffer CI
Change collection tube

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 (3.1.5) and incubate for 2 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, 2 min
9 ml Elution Buffer

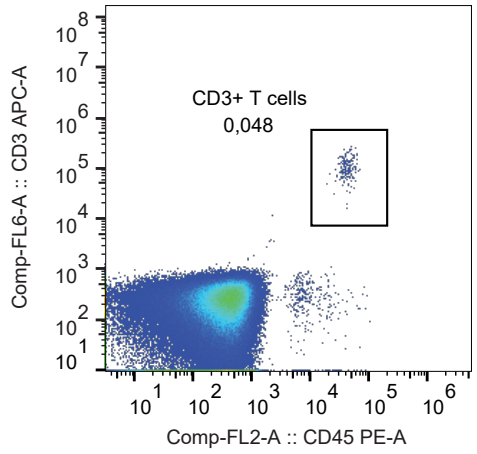
10 ml Elution Buffer



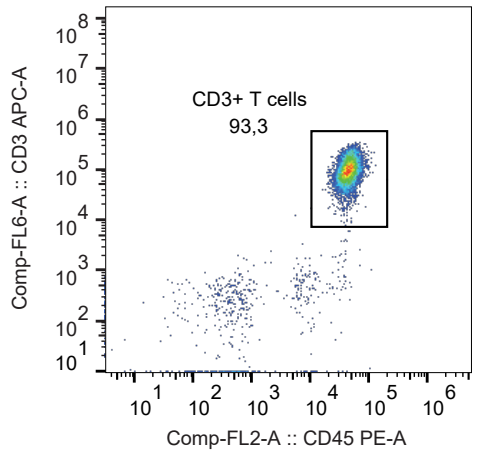
4. EXAMPLE ANALYSIS

Separation of $CD3^+$ T cells from 7.5 ml whole blood sample using the Fab-TACS Gravity CD3 Isolation Kit without flow restrictor. Unlysed cells were stained with CD3-APC (OKT-3) / CD45-PE (HI30) 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



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