

## Manual

# CD4 FAB-TACS® GRAVITY KIT

human, whole blood, cat. no. 6-6202-004 (4 columns) and 6-6202-010 (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, human, lyophilized  
6-8002-191 (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 containing 1 mM EDTA and 0.5% BSA.

## 3. PROTOCOL

### 3.1. Reagent preparation

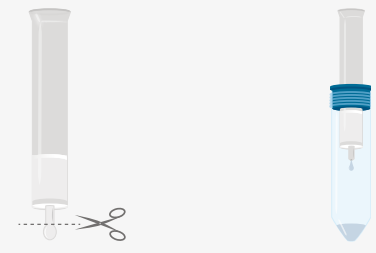
Allow the reagents to equilibrate to room temperature (RT) prior to use. For a sterile isolation, work under a safety cabinet. **The following volumes will be sufficient for one selection process.**

- 3.1.1. Prepare **1x Buffer CI** from 10x Buffer CI stock. Optional: degas buffer before use, as air bubbles could block the column..
- 3.1.2. Dissolve lyophilized Fab-Strep in 1 ml Buffer CI by carefully pipetting up and down. Do not vortex! Transfer 200 µl Fab-Strep solution **each** into a fresh reagent tube. **The 200 µl Fab-Strep aliquots may be stored at -20° C.**
- 3.1.3. Add 800 µl Buffer CI to 200 µl Fab-Strep solution. The Fab-Strep 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.
- 3.2.3. Apply the 1 ml Fab-Strep solution (3.1.3) onto the Fab-TACS® Gravity column. Let the Fab-Strep solution enter the packed bed completely. Incubate for 2 min.

**Prepare column**

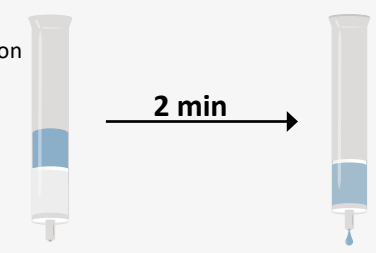


**Wash**

8 ml Buffer CI

**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



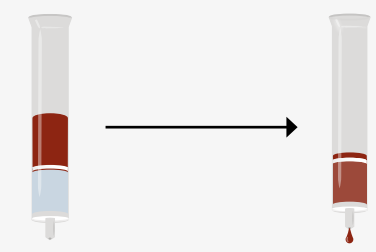
**3.3. Cell isolation with Fab-TACS® Gravity columns**

- 3.3.1. Loading – Apply diluted 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**

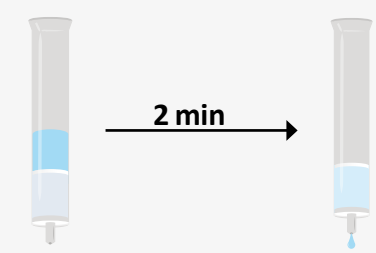
4x 10 ml Buffer CI  
Change collection tube



- 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 10 ml Biotin Elution Buffer.

**Elute**

1 ml Elution Buffer  
Incubate, 2 min  
10 ml Elution Buffer  
10 ml Elution Buffer

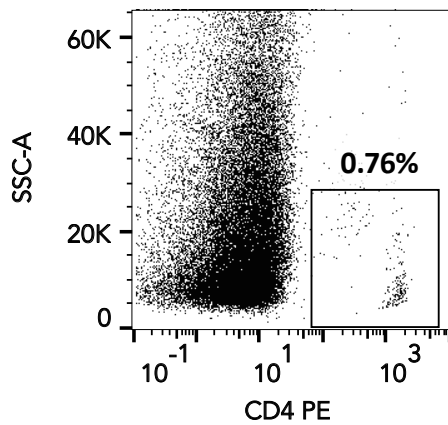


- 3.3.4. Elute with additional 10 ml Biotin Elution Buffer.

#### 4. EXAMPLE ANALYSIS

Separation of CD4<sup>+</sup> T cells from 7.5 ml whole blood sample using the Fab-TACS® Gravity CD4 Isolation Kit without flow restrictor. Unlysed cells were stained with CD4-PE (OKT-4) 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

