Cell Selection & Expansion

For research use only

Protocol

# CD19 Nano-TACS® Agarose Column Starter Kit

Cat. no. 6-3307-002

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 |
| 6-8507-150 | CD19 Nano-Strep, mouse, lyophilized, 50 µg | 1 | 20 µg |
| 6-6996-001 | Biotin stock solution, 100 mM, 250 µl | 1 | 60 µl |
| 6-6320-025 | 10x Buffer CI, 25 ml<br>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:** **2 x $10^7$ target cells** 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 Nano-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

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

## 2.1. Reagent preparation

**2.1.1.** Prepare 1x Buffer CI from 10x stock by diluting with dd$H_2O$. 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

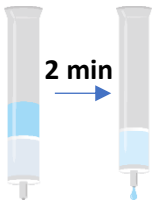
**2.2.1.** Prepare splenocytes in Buffer CI.

**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.



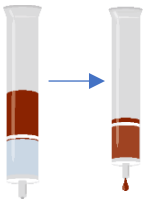

**2.3.3. Load** the **400 µl** Nano-Strep solution (2.1.2.) onto the Strep-Tactin® TACS Agarose Column. Let the Nano-Strep solution enter the packed bed completely. Incubate for **2 min**.

**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.**

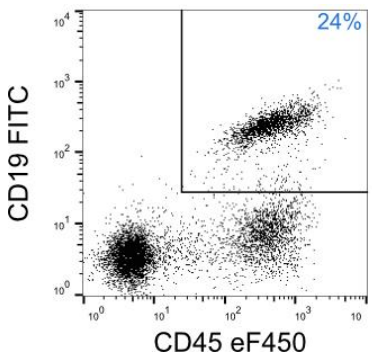
## 4. TROUBLESHOOTING

| | |
|---|---|
| **Low yield** | *Option 1:*<br>Check for biotin contamination in your samples.<br><br>*Option 2:*<br>Use flow restrictor during sample loading.<br><br>*Option 3:*<br>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. |

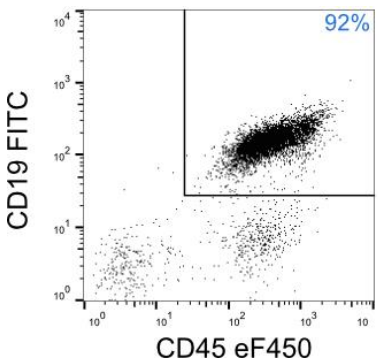
## 5. EXAMPLE DATA

Separation of CD19⁺ B cells from mouse spleen using the CD19 Nano-TACS® Agarose Column Starter Kit. Unlysed cells were stained with anti-mouse CD19-FITC (1D3) / CD45-eF450 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**



Watch this How-to video to see an exemplary isolation

**https://www.youtube.com/watch?v=bAaMgNlkTDI**

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If you have any questions, please contact

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We are here to help!



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