Cell Selection & Expansion

! For research use only

Protocol

CD3/CD28 Streptamer® Kit

Cat. no. 6-8900-050

human, for T cell expansion

1. GENERAL INFORMATION & TECHNICAL SPECIFICATIONS

Kit components:

Cat. no.	Product	Quantity
6-8910-125	CD3 Fab-Strep, human, lyophilized, 125 µg	1
6-8911-125	CD28 Fab-Strep, human, lyophilized, 125 µg	1
6-8912-050	Strep-Tactin® Multimer, 500 µl	1
6-6325-001	Biotin stock solution, 100 mM, 1 ml	1

Specifications: For stimulation and expansion of **5 x 10^7** T cells or PBMCs

Required: Appropriate cell culture medium (recommended: RPMI + 10% FCS + 1% Pen-Strep + 50U/ml IL-2); buffer (e.g. PBS)

Storage: Store all components at 2 - 8 °C. Store reconstituted Fab-Streps at -80 °C.

Stability: 6 months after shipping.

Shipping: Blue ice

Warnings: 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

Volumes are suitable for stimulation and expansion of **1 x 10^6** cells. For different cell numbers, adapt volumes according to **Table 1**.

2.1.1. Dissolve each Fab-Strep in **500 µl** buffer. Store Fab-Strep suspensions in aliquots at -80 °C.

2.1.2. Combine **10 µl** CD3 Fab-Strep with **10 µl** CD28 Fab-Strep and **10 µl** of Strep-Tactin® Multimer in a 0.5 ml tube. Incubate the mixture for at least **20 min at 4 °C** under constant agitation (e.g. using a roller mixer) to generate CD3/CD28 Streptamer® complexes.

We recommend preparing CD3/CD28 Streptamer® complexes freshly for each experiment. If necessary, store pre-mixed components at 4 °C. **Do not freeze!**

2.2. Cell preparation

2.2.1. Isolate primary T cells or subsets of interest from blood or buffy coat.

Alternatively, PBMCs can be used. Recommended cell numbers and volumes in every step are identical to pre-isolated T cells.

2.2.2. Resuspend cells in cell culture medium at a concentration of 0.2 x 10^6 - 1 x 10^6 cells per 1 ml (optimal conditions should be titrated).

Table 1: Recommended volumes for different cell numbers

	96-well	48-well	24-well
Cell number	5 - 8 x 10^4	2 - 5 x 10^5	0.5 - 1 x 10^6
Volume culture medium [ml]	0.1 - 0.2	0.5 - 1.0	1.0 - 2.0
CD3/CD28 Streptamer® premix [µl]	3	15	30
Biotin [µl]	1 - 2	5 - 10	10 - 20

Biotin is required if you want to terminate the stimulation at a certain point during the expansion period or remove CD3/CD28 Streptamers from the cells. See **3.3.** for details.

3. PROTOCOL

3.1. T cell activation

3.1.1. Seed **0.5 – 1 x 10^6** purified T cells in **1 – 2 ml** cell culture medium in a 24-well plate.

3.1.2. Add **30 µl** CD3/CD28 Streptamer® premix to the cells and mix gently. Incubate cell suspension in a humidified CO_2 incubator at 37 °C, according to your experimental setup.

3.1.3. Harvest activated T cells and use directly for further analysis.

Activation markers CD25 and CD69 should be upregulated after 48 h.

3.2. T cell expansion

3.2.1. Examine culture daily regarding cell size, shape and cluster formation (using a microscope). Count the cells (at least every two or three days) to evaluate cell density (should not exceed 2.5 x 10^6 cells/ml). If cell medium turns yellow or cell density is too high, split cultures back to a density of 0.2 - 1 x 10^6 cells/ml into a new plate of appropriate size.

3.2.2. Restimulation of the cells might be necessary after a couple of days in culture (signs of exhaustion typically after 7 - 10 days). Repeat therefore from step 3.1.2.

3.3. Termination of stimulation/removal of CD3/CD28 Streptamers

3.3.1. Add biotin directly to the culture medium containing the activated T cells (volumes see Table 1). The final concentration of biotin should be 1 mM.

3.3.2. Incubate cell suspension for **30 min at room temperature**.

3.3.3 Harvest cells by resuspension and transfer them to a 15 ml reaction tube. Add **10 ml** of culture medium for dissociation of CD3/CD28 Streptamers. Collect cells by centrifugation at **300 x g** for **6 – 10 min**.

3.3.4. **Discard supernatant completely** and repeat washing with **10 ml** cell culture medium. Cells are now ready for further downstream analyses

For T cell expansion, cells should be stimulated at least for 24 h.

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If you have any questions, please contact

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We are here to help!

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