REDCONJU · PRODUCT MANUAL

人 CD3/CD28 T 细胞激活磁珠试剂盒

Human CD3/CD28 T Cell Activation/Expansion Kit

货号	HXL-0000TA-1 / HXL-0000TA-2	规格	分别适用于约 1×10⊠ / 2×10⊠ 个起始 T 细胞或 PBMC
用途/方式	T 细胞激活与扩增	推荐用量	标准起点：25 µL 磁珠 / 5×10⊠ PBMC；按实验优化
适用样本	人 PBMC 或纯化 T 细胞	保存条件	2–8°C避光保存；禁止冷冻

仅供科研使用（RUO），不用于临床诊断或治疗。

1. Description

This product is for research use only.

Components: 1 mL ConjuFlex® Particles, cell culture grade, corresponding to 4×108 ConjuFlex® Particles; ConjuFlex® Particles conjugated to monoclonal streptavidin antibodies, (antibodies CD3 and CD28)

Product format

Storage All components are supplied with Low endotoxin. Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of T Cell Activation/Expansion Kit

The T Cell Activation/Expansion Kit has been designed to activate and, if required, expand human T cells. The ConjuFlex® Particles are used to mimic antigen-presenting cells and activate resting T cells from peripheral blood mononuclear cells (PBMCs) as well as purified T cells. T cell expansion is achieved by culturing and reactivation at day 14 of culture.

1.2 Applications

● Activation and expansion of resting T cells from PBMCs.

● Activation and expansion of T cells

● Expansion of antigen-specific T cells.

1.3 Reagent and instrument requirements

● Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% human serum albumin (HSA), and 2 mM EDTA. Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

▲ Note: HSA can be replaced by other proteins such as bovine serum albumin (BSA), fetal bovine serum (FBS), or human AB serum. Buffers or media containing Ca2+ or Mg2+ are not recommended for use.

● Medium: RPMI 1640 supplemented with 10% AB serum.

▲ Note: 2-Mercaptoethanol (0.01 mM) can be added to preserve cell viability in case of rapid cell growth.

● (Optional) Cell expansion bags or flat bottom cell culture plates with lids.

Humidified incubator

2. Protocol

All steps in the protocol have to be performed under sterile conditions.

2.1 T cell activation protocol

This T cell activation protocol is optimized for strong activation of PBMCs, using one ConjuFlex® Particles per two PBMCs or T cells (bead-to-cell ratio 1:2). The same bead-to-cell ratio is recommended when activating purified T cells.

▲ Note: Other ratios than 1:2 of loaded Streptavidin ConjuFlex® Particles per cell may be required for other applications (refer to 1.2 Background information).

▲ Volumes for activation given below are for up to 5×106 PBMCs. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g., for 1×107 total cells, use twice the volume of all indicated reagent volumes and total volumes).

For the activation of purified T cells, cultivate T cells at 2.5×106 cells per mL per cm2.

1. Resuspend ConjuFlex® Particles thoroughly and transfer 25 µL (2.5×106 loaded Streptavidin ConjuFlex® Particles) per 5×106 PBMCs to a suitable tube.

▲ Note: If unloaded ConjuFlex® Particles shall be used for negative control experiments, replace loaded Streptavidin ConjuFlex® Particles by adding

12.5 µL (2.5×106 beads) of unloaded Streptavidin ConjuFlex® Particles per 5×106 PBMCs.

2. Add 100–200 µL culture medium to the ConjuFlex® Particles and magnetic separator separates for 5 minutes.

Allow the ConjuFlex® Particles to adhere to the wall of the tube:

3. Aspirate supernatant and resuspend ConjuFlex® Particles in 100 µL fresh culture medium.

4. Resuspend PBMCs at a density of 5×106 cells per 900 µL of culture medium RPMI 1640 supplemented with 10% AB serum.

5. Add the prepared Streptavidin ConjuFlex® Particles from step3 to the 900 µL of cell suspension and mix well.

6. Add the mixture to a suitable cell culture vessel at a density of 5×106 cells per mL per cm2.

7. Incubate at 37 °C, 5–10% CO2 for up to 3 days.

▲ Note: Inspect cultures daily, and add fresh medium if required.

8. For T cell expansion, proceed to step 2 of the T cell expansion protocol (refer to section 2.2). For immunofluorescent staining proceed to 2.3.

2.2 T cell expansion protocol

As T cells are activated and expand, the addition of further growth factors and stimuli, such as more loaded Streptavidin ConjuFlex® Particles is required. The following procedure is a guideline for the stimulation and expansion ofT cells.

1. Perform steps 1–7 as described under section 2.1 (T cell activation protocol).

2. At day 3 gently pipette cell suspension up and down to breakup clumps.

3. Split cell suspension into two equal parts and add culture medium supplemented with 20 units rIL-2 per mL. Incubate at 37 °C, 5–10% CO2.

▲ Note: If rIL-2 interferes with downstream experiments, it may be omitted. However, omission will lower the cell viability.

4. At day 7 gently pipette cell suspension up and down to break up clumps. Count T cells and dilute to 1.2–2.5×106 T cells per mL by adding fresh medium supplemented with 20 units rIL-2 per mL.

5. At day 11, split cell suspension into two equal parts and add fresh medium supplemented with 20 units rIL-2 per mL.

6. At day 14, resuspend T cells at 2.5×106 cells per mL in fresh medium supplemented with 20 units rIL-2 per mL.

7. For longer expansion, cells are now restimulated by adding one ConjuFlex® Particle per two cells: Count cells and add 12.5 µL ConjuFlex® Particles per 2.5×106 T cells.

8. Further cultivate cells and repeat steps 2 and 3 every 3–4 days.

▲ Note: Daily inspect culture. Depending on the expansion rate, it might be necessary to split culture more frequently than every 3–4 days.

9. Proceed to downstream application, e.g., analysis of cells.

▲Note: Removal of Streptavidin ConjuFlex® Particles is not required for immunofluorescent staining. For assays where T cells are required to return to a fully resting state before further stimulus, Streptavidin ConjuFlex® Particles should be removed at least 24 hours before restimulation (refer to section 2.4).

2.3 Immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for 106 total cells. When working with fewer than 106 cells and up to 107 cells, use the same volumes as indicated.

▲ ConjuFlex® Particles show no autofluorescence and do not need to be removed prior to flow cytometric analysis.

▲ After short term stimulus for up to 24 hours, scatter properties of cells may be altered due to strong interaction between cells and ConjuFlex® Particles.

▲ Upon stimulation, expression of CD3 might be transiently down- regulated. Thus, the staining of CD3 on the cell surface of activated cells might be affected.

1. Resuspend cells to break up cell clumps.

▲ Note: In short-term stimulus of cells (up to 24 hours), the Streptavidin ConjuFlex® Particles may be bound strongly to cells. Care should be taken to thoroughly resuspend cells before analysis.

2. Wash cells by adding 1–2 mL of buffer per 106 cells and centrifugeat 300×g for 10 minutes. Aspirate supernatant completely.

3. Add staining antibodies, e.g., CD4-FITC and CD25-PE to 106cells resuspended with buffer according to the manufacturer’ s recommendations.

4. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

▲ Note: Higher temperatures and/or longer incubation times lead to non-specific cell labeling. Working on ice requires increased incubation time.

5. Wash cells by adding 1–2 mL of buffer per 106 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

6. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

2.4 Removal of ConjuFlex® Particles

▲ Removal of ConjuFlex® Particles may be required before magnetic separation of cells with other magnetic beads (MACS microbeads or Stemcell beads) or before restimulation with different agents or antigens.

1. Harvest cells and transfer to a 5 mL, 13–15 mL, or 50 mL tube and wash once with buffer.

2. Resuspend cells in buffer at a density of up to 2×107 cells per1 mL and vortex thoroughly.

3. Place the tube in the magnetic field of the magnetic Separator.

▲ Note: Use the Tube Rack to insert 5 mL tube into the magnetic field of the separator. For details refer to the Magnetic Separator data sheet.

4. Allow the ConjuFlex® Particles to adhere to the wall of the tube: 5 mL tubes: 5 minutes 13–15 mL or 50 mL tubes: 10 minutes

5. Retaining the tube in the Magnetic Separator, carefully pipette of the supernatant containing the ConjuFlex®-depleted cells into a new tube.

6. Remove the tube from the separator and add buffer to the same volume as before.

7. Vortex sample, replace tube in the Magnetic Separator, and repeat steps 4–5.

8. Collected cells can now be further processed as required.