Miltenyi Biotec

T Cell TransAct™
human

Order no. 130-111-160

Contents

1. Description

This product is for research use only.

Components	2×2 mL T Cell TransAct™, human
Capacity	The reagent is sufficient to activate and expand up to 4×10^8 enriched T cells or up to 8×10^8 peripheral blood mononuclear cells (PBMCs), when used at recommended titer of 1:100.
Product format	Polymeric nanomatrix conjugated to humanized CD3 and CD28 agonist supplied in phosphate-buffered-saline (PBS), containing 0.03% poloxamer 188 as stabilizer, pH 7.3–7.9.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

The T Cell TransAct has been designed to activate and expand enriched T cell populations or human resting T cells from peripheral blood mononuclear cells (PBMCs). T cell expansion is achieved by culturing for up to 14 days. For longer cultivation restimulation after 14 days is necessary.

Polyclonal T cell expansion can be used when increased numbers of T cells are required or when T cells are activated to enable gene modification.

Due to the nanomatrix of the T Cell TransAct, it can be sterile filtered and excess reagent can be removed by simple replacement of supernatant or by a washing step, e.g., centrifugation.

The recommended titers have been found to efficiently stimulate the majority of T cell subsets, however, for special applications it is recommended to experimentally determine the optimal stimulation titer. Over-activation of T cells carries a risk of activation-induced cell death.

The T Cell TransAct has been developed in combination with the TexMACS™ Medium and Human IL-2 IS or Human IL-7 and Human IL-15.

1.2 Applications

- The T Cell TransAct is intended for the *in vitro* stimulation and expansion of purified T cell populations of, for example, untouched T cells isolated with the Pan T Cell Isolation Kit, human, as well as of human T cells from hematological cell populations (e.g. PBMCs).

1.3 Reagent and instrument requirements

- TexMACS Medium, research grade (# 130-097-196) supplemented with Human IL-2 IS, premium grade (# 130-097-744) or Human IL-7, premium grade (# 130-095-361) and Human IL-15, premium grade (# 130-095-762).
- Buffer for flow cytometric analysis: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD4-VioBlue®, CD8-VioGreen™, CD25-PE, and CD69-APC. For more information about fluorochrome-conjugated antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Pan T Cell Isolation Kit, human (# 130-096-535)
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of ded cells.

2. Protocol

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

▲ Excess of T Cell TransAct is removed by simple replacement of supernatant or by a washing step, e.g., centrifugation (at least 10-fold reduction) 2–3 days after initial stimulation. Performing a washing step earlier may result in reduced T cell proliferation.

▲ Activated T cells can be transduced 1–2 days after activation. The optimal virus titer has to be defined before and depends on the viral vector used. The T Cell TransAct can be used in combination with retro- or lenti-viral transduction.

▲ Presence of residual EDTA, e.g. when using medium containing EDTA for T cell purification, will hamper T cell stimulation. Ensure extensive removal of EDTA (i.e. over 200-fold reduction) prior to T cell stimulation with the T Cell TransAct.

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140-004-952.03

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

For the isolation of purified T cells use, for example, the Pan T Cell Isolation Kit, human.

2.2 T cell activation and expansion

The protocol has been optimized for gentle and efficient activation and expansion of purified T cells and PBMCs by using a titer of 1:100.

Purified T cells should be activated at an optimal surface density of 1×10^6 cells per cm^2 (table 1) and PBMCs with up to 2×10^6 per cm^2.

Culture plate	Growth area	Max. working volume	Total T cell number	T Cell TransAct to add per well
96 well	0.31 cm^2	0.2 mL	0.3×10^6	2 µL
48 well	1 cm^2	1 mL	1×10^6	10 µL
24 well	2 cm^2	2 mL	2×10^6	20 µL
12 well	4 cm^2	4 mL	4×10^6	40 µL
6 well	10 cm^2	5 mL	5×10^6	50 µL

Table 1: Optimal surface densitiy when working with purified T cells.

Volumes given below are for the stimulation in a 48-well plate of up to 1×10^6 purified T cells or up to 2×10^6 PBMCs in a total volume of 990 µL TexMACS™ Medium supplemented with 20 IU/mL Human IL-2 or 155 U/mL Human IL-7 and 290 U/mL Human IL-15. When working with fewer than 10^6 cells, use the same volumes as indicated in table 1. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

Activation in a 48-well plate

1. Determine cell number.
2. Resuspend cells in 990 µL supplemented TexMACS Medium.
3. Add 10 µL of the T Cell TransAct™.
4. Incubate at 37 °C, 5% CO_2 for up to 3 days.
   ▲ **Note:** Inspect culture daily, and add fresh medium if required.
5. Remove residual reagent 2–3 days after initial activation by either replacing 900 µl of supernatant with fresh supplemented TexMACS Medium or by centrifugation at 300×g for 10 minutes and aspirate supernatant completely.
6. Add 1 mL fresh supplemented TexMACS Medium and incubate at 37 °C, 5% CO_2.

Expansion

1. Split cell suspension every 2 days into two equal parts and add fresh supplemented TexMACS Medium.
2. Incubate at 37° C, 5% CO_2.
   ▲ **Note:** For optimal expansion of T cells a daily inspection of culture is required. It might be necessary to split culture more or less frequently than every day.
3. At day 14 proceed to downstream application, e.g., analysis of cells.
4. (Optional) T cells can be further expanded by reapplying T Cell TransAct to the culture. However, for restimulation it is recommended to use a titer of 1:500.

2.3 Immunofluorescent staining

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ It is recommended to stain 10^6 cells per sample. When working with up to 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

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

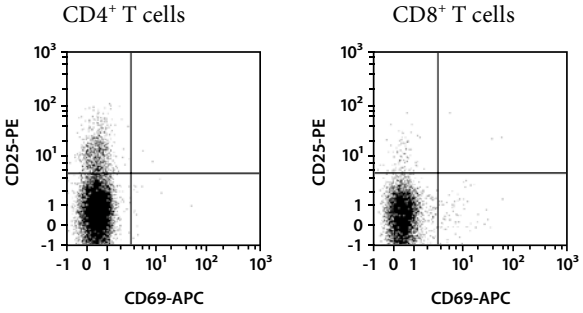
1. Determine cell number.
2. Wash cells by adding 1–2 mL of buffer per 10^6 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Add each staining antibody, e.g., CD4-VioBlue®, CD8-VioGreen™, CD25-PE, and CD69-APC according to manufacturer's recommendations.
4. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
5. Wash cells by adding 1–2 mL of buffer per 10^6 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.

3. Examples of T cell activation and expansion using the T Cell TransAct™

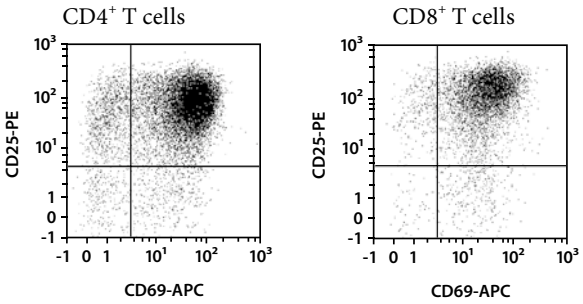
A) Example of a T cell activation

Human purified T cells were isolated using the Pan T Cell Isolation Kit and activated for 48 hours using the T Cell TransAct™ (titer 1:100) in TexMACS™ Medium supplemented with Human IL-2 (20 IU/mL). The negative control experiment was performed without adding the T Cell TransAct. Cells were fluorescently stained using CD25-PE and CD69-APC and analyzed by flow cytometry using the MACSQuant® Analyzer. CD4-VioBlue® was used for selection of T helper cells and CD8-VioGreen™ was used for selection of cytotoxic T cells. Dead cells and debris were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

Negative control

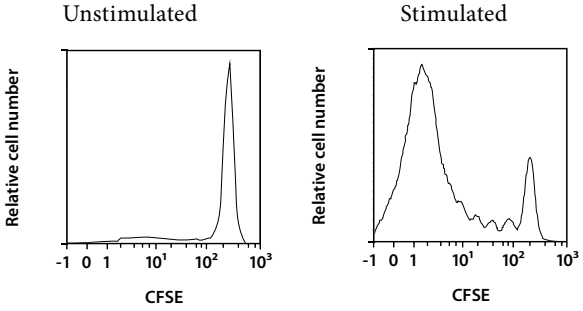


T cells after activation



B) Expansion of pan T cells after activation

Isolated Pan T cells were labeled with carboxyfluorescein succinimidyl ester (CFSE) and stimulated with the T Cell TransAct. T cells were cultured at a density of 1×10^6 cells per cm^2 in supplemented TexMACS Medium supplemented with Human IL-2 (20 IU/mL). Proliferation analysis was done by flow cytometry via the detection of the CFSE dilution 7 days after stimulation. Non-stimulated pan T cells act as negative control.



Refer to **www.miltenyibiotec.com** for all data sheets and protocols.

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140-004-952.03