Miltenyi Biotec

CytoStim™
non-human primate

200 µL	130-094-447
1 mL	130-094-442

Contents

1. Description

This product is for research use only.

Components	200 µL CytoStim™, non-human primate, or 1 mL CytoStim™, non-human primate
Capacity	200 µL for stimulation of 10^8 total cells, or 1 mL for stimulation of 5×10^8 total cells.
Product format	CytoStim is supplied in buffer containing stabilizer.
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

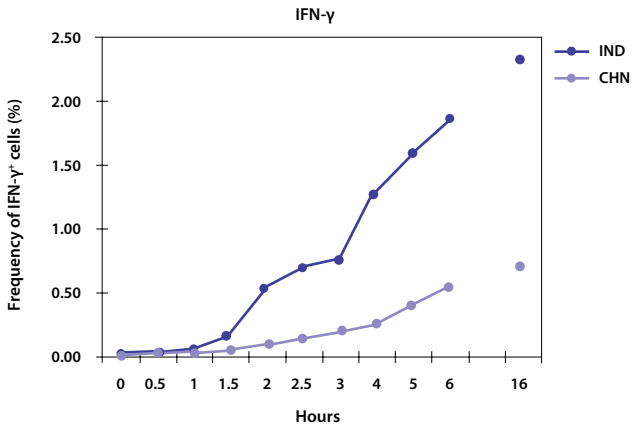
CytoStim has been developed for rapid and efficient restimulation of non-human primate effector/memory T cells. CytoStim causes activation of T cells by binding the T cell receptor (TCR) and crosslinking it to a major histocompatibility complex (MHC) molecule of an antigen-presenting cell (APC). CytoStim is an antibody-based reagent that acts similar to a superantigen but independently of certain Vβ domains of the TCR. Upon stimulation with CytoStim, CD4$^+$ and CD8$^+$ cells start to secrete effector cytokines or up-regulate activation markers on their cell surface within a few hours.

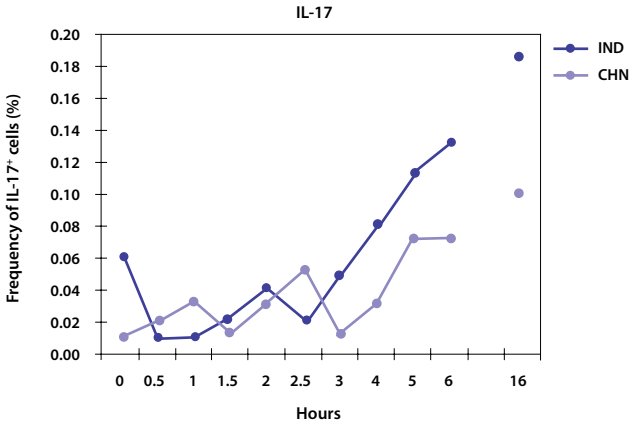
1.2 Applications

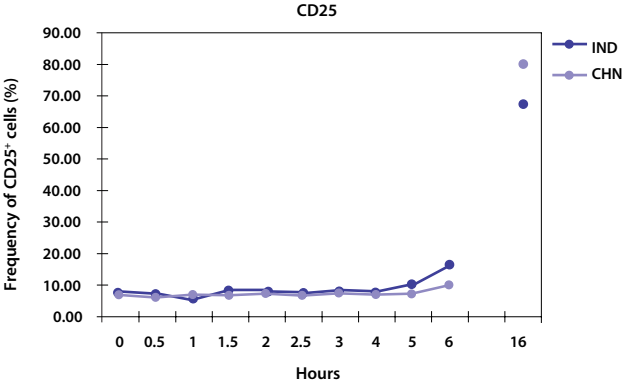
- Rapid stimulation of T cells as a positive control for cytokine expression.
- Rapid stimulation of T cells as a positive control for expression of activation markers.

1.3 Kinetics of cytokine and activation marker expression upon stimulation with CytoStim

The following expression kinetics of IFN-γ, IL-17, CD25, CD69, and CD154 upon stimulation with CytoStim were determined from two species, indian (IND) and chinese (CHN) rhesus monkey. Figures show frequencies of IFN-γ$^+$, IL-17$^+$, CD25^{++}, CD69$^+$, and CD154$^+$ cells among viable CD4$^+$ lymphocytes.







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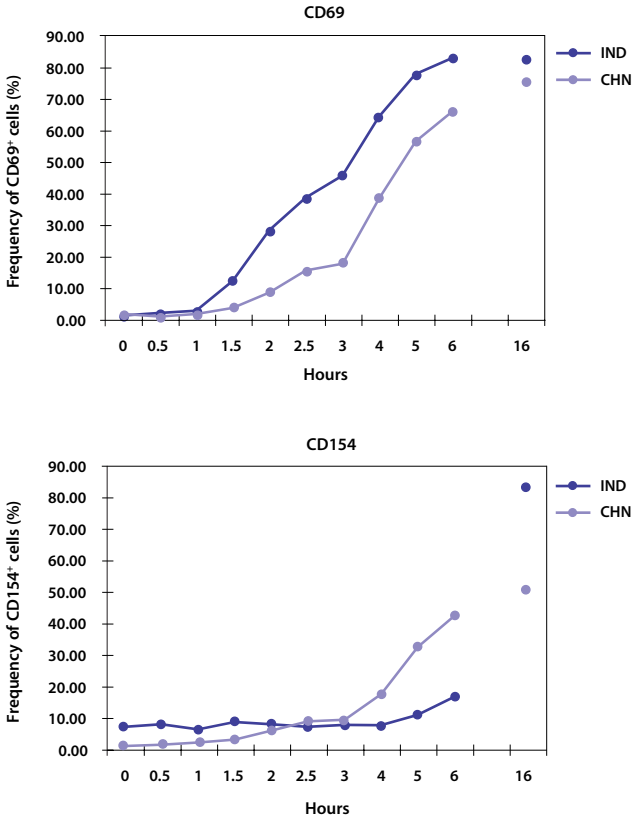


2. Recommendations for *in vitro* stimulation of T cells with CytoStim™

2.1 Reagent requirements

- Culture medium, e.g., RPMI 1640 with stable glutamine supplemented with 5% non-human primate serum or 10% fetal bovine serum (FBS).
- (Optional) Intracellular cytokine staining, e.g., with Anti-IFN-γ-PE or Anti-IL-17-PE. For additional reagent requirements refer to the respective data sheet.
- (Optional) Surface staining reagents, such as CD69-FITC, CD25-PE, CD154-PE, or CD154-APC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

  ▲ **Note:** If CD154 antibodies are used in the labeling step of the cytokine secretion assay to stain activated $CD4^+$ T cells, a CD40-blocking antibody has to be added during the *in vitro* stimulation step to prevent CD154 down-regulation.

2.2 Sample preparation

For activation of T cells, best results are achieved by stimulation of freshperipheral blood mononuclear cells (PBMCs), whole blood, or other leukocyte containing single-cell preparations from tissues or cell lines. Alternatively, frozen cell preparations can be used.

When working with anticoagulated peripheral blood or buffy coat, 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.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

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

It is necessary, that the cell preparation also contains APCs for efficient stimulation of the T cells. When working with purified T cells, APCs need to be added to the culture.

▲ **Note:** PBMCs may be stored overnight. The cells should be resuspended and incubated in culture medium as described in 2.3 steps 1–3 without addition of CytoStim. CytoStim is then added to the culture on the next day.

2.3 *In vitro* stimulation of T cells

▲ Always include a negative control in experiment. The sample should be treated exactly the same as the stimulated sample, except for the addition of CytoStim.

▲ A positive control should also be included in experiment, stimulated with CytoStim.

▲ Do not use media containing any foreign proteins, such as BSA or FBS, because of non-specific stimulation.

1. Wash cells by adding medium and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cells in culture medium at 10^7 cells/mL. Plate cells in dishes at a density of 5×10^6 cells/cm^2 (refer to 4. Appendix: Flask and dish sizes for *in vitro* stimulation of T cells).
3. Add 20 µL of CytoStim per mL cell suspension. Mix carefully and incubate cells at 37 °C; 5% CO_2.

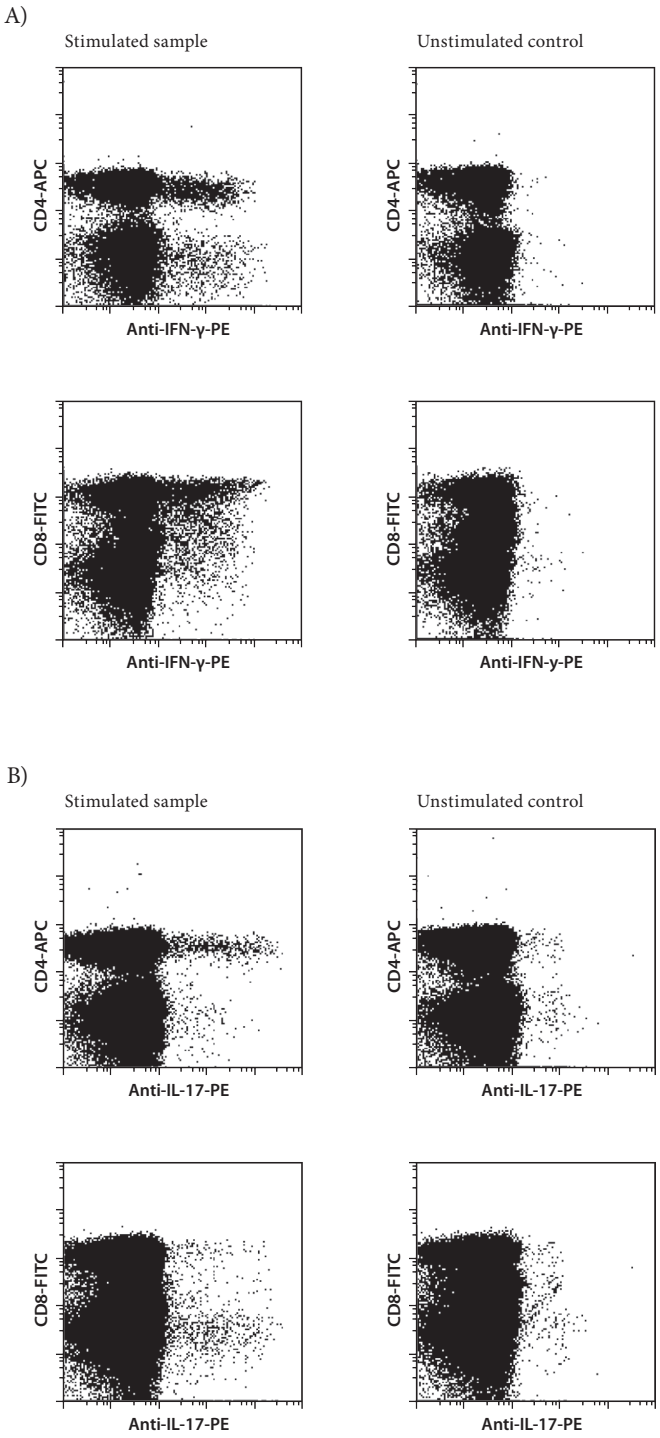
   Intracellular cytokine staining: Incubate cells for 2 hours, then add 1 µg/mL brefeldin A, and incubate for further 4 hours.
4. Collect cells carefully by using a cell scraper or by pipetting up and down when working with smaller volumes. Rinse the dish with cold buffer. Check microscopically for any remaining cells; if necessary, rinse the dish again.

   Intracellular cytokine staining: When working with fluorochrome-conjugated MACS® anti-cytokine antibodies, refer to the protocol section of the respective data sheet.

   ▲ **Note:** When preparing cells for intracellular cytokine staining, fixed cells may be stored at 2–8 °C for up to one week.

3. Example of detection of IFN-γ$^+$ and IL-17$^+$ T cells upon restimulation with CytoStim™

PBMCs from rhesus monkeys were incubated with CytoStim™ and intracellulary stained with Anti-IFN-γ-PE, CD4-APC, and CD8-FITC (A) or with Anti-IL-17-PE, CD4-APC, and CD8-FITC (B) and analyzed by flow cytometry using the MACSQuant® Analyzer.



4. Appendix: Flask and dish sizes for *in vitro* stimulation of T cells

For *in vitro* stimulation of T cells (see 2.3) the cells should be resuspended in culture medium, containing 5% of non-human primate serum, at a dilution of 10^7 cells/mL. The cells should be plated at a density of 5×10^6 cells/cm². Both the dilution and the cell density are important to assure optimum stimulation.

The following table lists culture plate, dish and flask sizes suitable for different cell numbers. It also indicates the appropriate amount of medium to add.

Total cell number	Medium volume to add	Culture plate	Well diameter
0.15×10^7	0.15 mL	96 well	0.64 cm
0.50×10^7	0.50 mL	48 well	1.13 cm
1.00×10^7	1.00 mL	24 well	1.60 cm
2.00×10^7	2.00 mL	12 well	2.26 cm
5.00×10^7	5.00 mL	6 well	3.50 cm
Total cell number	**Medium volume to add**	**Culture dish**	**Dish diameter**
4.5×10^7	4.5 mL	small	3.5 cm
10.0×10^7	10.0 mL	medium	6 cm
25.0×10^7	25.0 mL	large	10 cm
50.0×10^7	50.0 mL	extra large	15 cm
Total cell number	**Medium volume to add**	**Culture flask**	**Growth area**
12×10^7	12 mL	50 mL	25 cm²
40×10^7	40 mL	250 mL	75 cm²
80×10^7	80 mL	720 mL	162 cm²
120×10^7	120 mL	900 mL	225 cm²

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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