DATA SHEET

CytoStim

Code Number#KT130-092-173

Background Information
CytoStim has been developed for rapid and efficient restimulation of human effector/memory T cells. CytoStim causes activation of T cells by binding the T cell receptor (TCR) and crosslinking it to an 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+ activation markers on their cell surface within a few hours.

Applications
Rapid stimulation of T cells as a positive control for cytokine expression.

Frequencies of cytokine-producing cells upon stimulation with CytoStim

Frequencies of the following cytokine-producing cells among $CD4^+$ cells were determined after one hour stimulation with CytoStim™ in human pheripheral blood mononuclear cells (PBMCs) using MACS® Cytokine Secretion Assays:

IFN-γ	0.12–9.10%
IL-2	0.08–0.24%
IL-4	0.02–0.04%
IL-5	0.02–0.14%
IL-10	0.15–0.20%
TNF-α	1.00–1.50%

For IL-17–secreting $CD4^+$ T cells the maximun frequency was determined after four hours stimulation with CytoStim in human PBMCs using MACS Cytokine Secretion Assays:

IL-17A	0.60–1.40%

Recommendations for *in vitro* stimulation of T cells with CytoStim
Reagent requirements
● Culture medium, e.g., TexMACS™ Medium (# 130-097-196) supplemented with 5% human serum.
Note: When using CytoStim™ as a positive control for T cells stimulated with specific antigens, avoid using non-human proteins such as bovine serum albumin (BSA) or fetal bovine serum (FBS). Autologous or human AB serum is recommended.
• (Optional) MACS Cytokine Secretion Assay Kit. For additional reagent and instrument requirements refer to the respective data sheet. For a detailed product list of MACS Cytokine Secretion Assays refer to www.miltenyibiotec.com.
• (Optional) Intracellular cytokine staining, e.g., with Anti-IFN-γ-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.

Sample preparation
For activation of T cells, best results are achieved by stimulation of fresh 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, 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.
When working with tissues or lysed blood, prepare a single-cell
suspension using standard methods. For details refer to the 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.

***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 non-human proteins, such as BSA or FBS, because of non-specific stimulation.
Wash cells by adding medium and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
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).
Add 20 µL of CytoStim per mL cell suspension. Mix carefully and incubate cells at 37 °C; 5% CO2.

Cytokine Secretion Assay: Incubate cells for 1–4 hours, depending on the cytokines to be analyzed.
Note: Cells can be prepared and placed into culture overnight without addition of CytoStim. CytoStim is then added the next morning for 1–4 hours of stimulation, directly followed by the Cytokine Secretion Assay.

Intracellular cytokine staining: Incubate cells for 2 hours, then add 1 µg/mL brefeldin A, and incubate for further 4 hours.
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.

Cytokine Secretion Assay: Refer to the protocol section of the respective data sheet.

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.

Storage: Store protected from light at 2−8 °C. Do not freeze.

Expiration Date: The expiration date is indicated on the vial label.

For In-Vitro Diagnostic Use. Not For Human Or Animal Consumption