Miltenyi Biotec

# CytoBox Th17
## mouse

Order no. 130-107-758

## Contents



## 1. Description

**This product is for research use only.**

**Components**

CytoBox Th17, mouse containing the following products:

| Product | Content | Order no. |
|---|---|---|
| Mouse IL-6, premium grade | 10 µg | 130-096-682 |
| Mouse IL-23, research grade | 5 µg | 130-096-676 |
| Mouse IL-1β, premium grade | 10 µg | 130-101-681 |
| Human TGF-β1, premium gade | 5 µg | 130-095-067 |
| Anti-IL-4 pure – functional grade, mouse | 500 µg in 0.5 mL | 130-095-709 |
| Anti-IFN-γ pure – functional grade, mouse | 500 µg in 0.5 mL | 130-095-729 |
| Anti-IL-2 pure – functional grade, mouse | 500 µg in 0.5 mL | 130-095-736 |

**Storage**

Lyophilized Mouse IL-6, premium grade, Mouse IL-23, research grade, Mouse IL-1β, premium grade, and Human TGF-β1, premium grade should be stored at –20 °C. Upon reconstitution aliquots should be stored at –20 °C or below. Avoid repeated freeze-thaw cycles.

Anti-IL-4 pure – functional grade, Anti-IFN-γ pure – functional grade, and Anti-IL-2 pure – functional grade should be stored at 2–8 °C protected from light. Do not freeze.

The expiration date is indicated on the vial label.

**Reconstitution**

It is recommended to reconstitute lyophilized Mouse IL-6, premium grade, Mouse IL-23, research grade, and Mouse IL-1β, premium grade with deionized sterile-filtered water to a final concentration of 0.05–1.0 mg/mL in a minimal volume of 100 µL, and Human TGF-β1, premium grade with 250 µL of deionized sterile-filtered water. Further dilutions should be prepared with 0.1% bovine serum albumin (BSA) or human serum albumin (HSA) in phosphate-buffered saline.

For technical specifications of the single components, please refer to the respective data sheet available at **www.miltenyibiotec.com/ds/*order_number*,** for example, for the data sheet of the Mouse IL-6 go to **www.miltenyibiotec.com/ds/130-096-682.**

### 1.1 Background information

The different T helper (Th) cell subsets have a central function in initiation, programming, and regulation of the various protective and pathological antigen-specific immune responses. T helper 17 (Th17) cells are responsible of the immune response against extracellular bacteria and fungi at the level of epithelial and mucosal tissues via secretion of IL-17, which activates neutrophils, and IL-22, responsible for several anti-microbial actions. However, Th17 cells are predominantly investigated for their role in autoimmune disorders, such as Crohn's disease, multiple sclerosis, and psoriasis, as well as in inflammatory diseases caused by persistent secretion of Th17 cytokines.

Th17 cells differentiate from naive T cells, but the conditions for Th17 cell polarization *in vivo* are still under investigation. *In vitro*, a combination of several cytokines is needed for Th17 polarization, including TGF-β, IL-6, IL-1β, and IL-23. Th17 differentiation can be further sustained with Anti-IFN-γ, Anti-IL-4, and Anti-IL-2 pure functional grade antibodies by blocking Th1 and Th2 polarization.

### 1.2 Applications

- *In vitro* polarization of mouse naive T cells into Th17 cells.

### 1.3 Reagent and instrument requirements

- Naive $CD4^+$ T Cell Isolation Kit, mouse (# 130-104-453)
- T Cell Activation/Expansion Kit, mouse (# 130-093-627)
- TexMACS™ Medium, research grade, (# 130-097-196) supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and 0.01 mM 2-Mercaptoethanol.
- (Optional) Spleen Dissociation Kit, mouse (# 130-095-926)
- (Optional) gentleMACS™ Octo Dissociator (# 130-095-937)


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- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, for example, CD4-VioBlue®, IFN-γ-APC, IL-4-PE, IL-17A-FITC, and RORγ (t)-APC. For more information about fluorochrome-conjugated antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for the flow cytometric exclusion of dead cells.
- (Optional, for analysis of secreted cytokines) MACSPlex Cytokine 10 Kit, mouse (# 130-101-740)
- (Optional, for enrichment of TH17 cells) Mouse Il-17 Secretion Assay – Cell Enrichment and Detection Kit (PE) (# 130-094-213)
- (Optional, for intracellular staining) Inside Stain Kit (# 130-090-477)

## 2. Protocol

### 2.1 Preparation of cells

1. Prepare a suspension of mouse splenocytes. Highly viable splenocytes can be obtained using the Spleen Dissociation Kit, mouse in combination with the gentleMACS™ Octo Dissociator.
2. Isolate mouse naive $CD4^+$ cells from splenocytes using the Naive $CD4^+$ T Cell Isolation Kit, mouse.

### 2.2 Polarization of naive T cells

1. Prepare cell culture medium by adding cytokines and antibodies to the supplemented TexMACS™ Medium as followed:

   20 ng/mL (10.000 U/mL) Mouse IL-6
   10 ng/mL (40 U/mL) Mouse IL-23
   10 ng/mL (8400 U/mL) Mouse IL-1β
   2 ng/mL (10 U/mL) Human TGF-β1
   10 µg/mL Anti-IL-4 pure – functional grade
   10 µg/mL Anti-IFN-γ pure – functional grade
   10 µg/mL Anti-IL-2 pure – functional grade

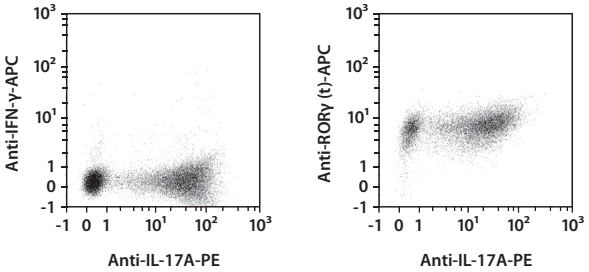
   ▲ **Note:** Refer to section 4.1 for convertion from U/mL to ng/mL.
2. Load Anti-Biotin MACSiBead™ Particles from the T Cell Activation/Expansion Kit with CD3ε-Biotin and CD28-Biotin as indicated in the data sheet.
3. Determine mouse naive $CD4^+$ cell number after isolation.
4. Resuspend cells at a density of $2\times10^6$ cells per mL of supplemented TexMACS Medium.
5. Add the cell suspension and the prepared Anti-Biotin MACSiBead Particles from step 2 to a suitable cell culture vessel at a density of $1\times10^6$ cells per mL per $cm^2$ (e.g. $0.25\times10^6$ cells in 250 µL/well of a 96-well plate).

   ▲ **Note:** Refer to 4.2: Plate sizes for *in vitro* T cell polarization.
6. Incubate at 37 °C and 5–10% $CO_2$ for up to 6 days.

   ▲ **Note:** Inspect cultures daily, and add fresh supplemented TexMACS Medium if required.
7. At day 2, gently pipette culture up and down to break up all cell clumps.
8. Split the cell culture every two days 1:4 or 1:2, depending on the proliferation of cells, and add fresh supplemented TexMACS Medium.
9. After 6 days of cultivation, polarized TH17 cells can be further processed for downstream analysis, for example, intracellular cytokine staining. Resting T cells require a restimulation for further expansion or analysis.

## 3. Example of TH17 cell generation using the CytoBox TH17

Naive T cells were cultivated in supplemented TexMACS Medium. $0.25\times10^6$ enriched naive $CD4^+$ T cells and $0.75\times10^6$ prepared Anti-Biotin MACSiBeads Particles were added in a 96-well flat bottom plate and split 1:2 on day 3 and 4. At day 5 cells were restimulated with PMA/ionomycin for 5 hours, with brefeldin A added for the last 4 hours. Cells were fluorescently stained with Anti-IL-17-PE and Anti-IFN-γ-APC as well as with Anti-IL-17-PE and Anti-RORγ (t)-APC for expression of lineage-specific transcription factors and analyzed by flow cytometry using the MACSQuant® Analyzer. Gating was perfomered on live $CD4^+$ cells.



# 4. Appendix

## 4.1 Calculation of cytokine concentration

In order to obtain maximal reproducibility for your TH17 differentiation experiments, it is recommended to always dose recombinant cytokines at a defined unit dose in [U/mL].
Lot-specific biological activities for premium grade cytokines are stated on the Certificate of Analysis (CoA), provided by the Technical Support upon request.

To calculate the cell culture concentration in [ng/mL] corresponding to the concentration in [U/mL], apply the following formula:

**Example for Human TGF-β1**

$$\text{Final culture concentration in [ng/mL]} = \frac{10\ \text{U/mL}}{\text{biological activity in [U/mg]*}} \times 10^6$$

* Please refer to corresponding data sheet or CoA to obtain the biological activity.

## 4.2 Plate sizes for *in vitro* T cell polarization

For T cell polarization the cells should be resuspended in culture medium at $1\times10^6$ cells/mL. The cells should be plated at a density of $1\times10^6$ cells/cm². Both the dilution and the cell density are important to assure optimal stimulation and cell growth.

The following table lists culture plate 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 |
|---|---|---|---|
| $0.25\times10^6$ | 0.25 mL | 96 well | 0.64 cm |
| $1.00\times10^6$ | 1.00 mL | 48 well | 1.13 cm |
| $2.00\times10^6$ | 2.00 mL | 24 well | 1.60 cm |
| $4.00\times10^6$ | 4.00 mL | 12 well | 2.26 cm |
| $10.00\times10^6$ | 10.00 mL | 6 well | 3.50 cm |

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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