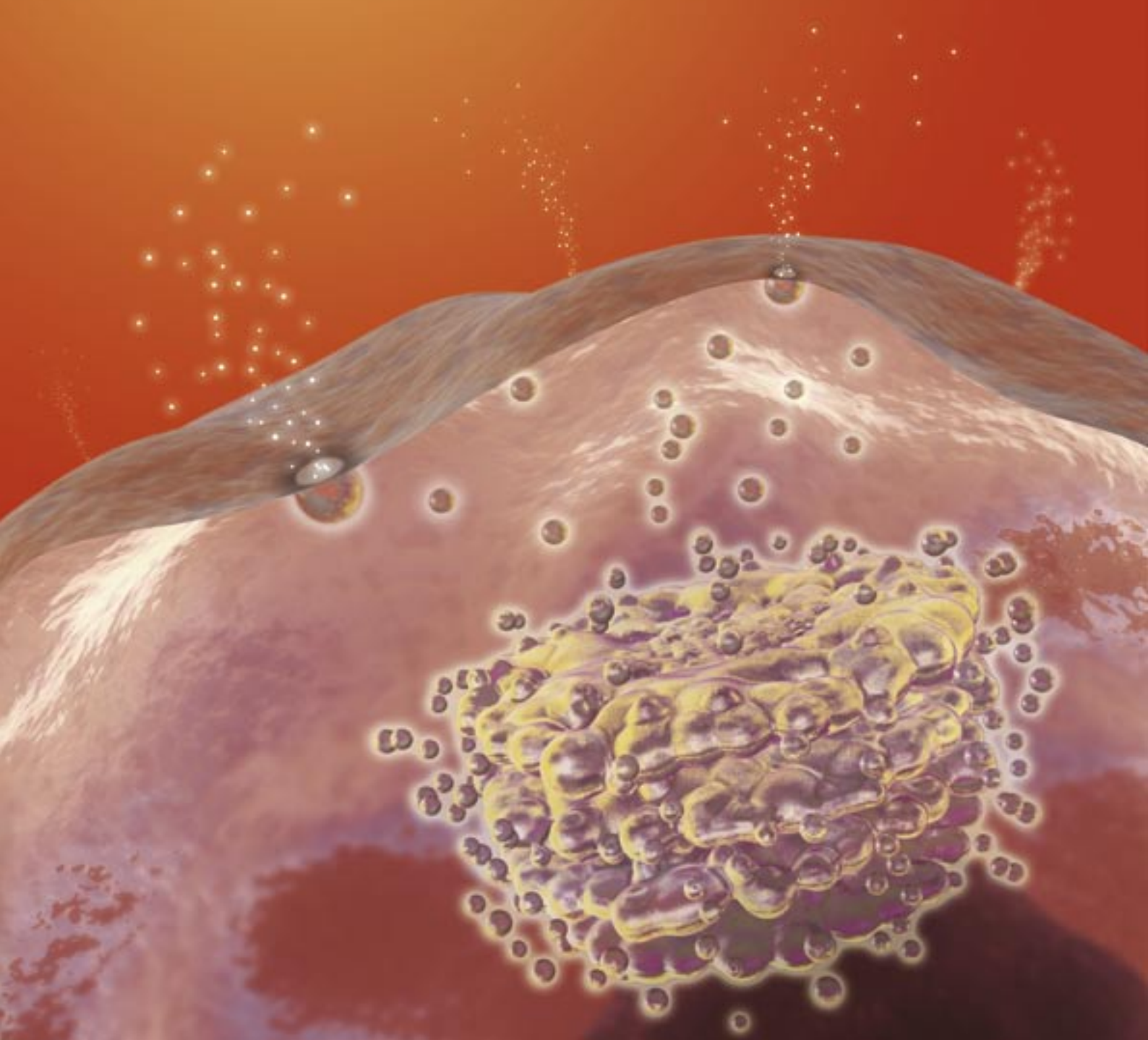




Miltenyi Biotec

# Analysis and isolation of cytokine-secreting cells

Antigen-specific T cells • Regulatory T cells • Dendritic cells  
NK cells/NKT cells • B cells • Other leukocytes



# Table of contents

<b>1. MACS® Products for cytokine-producing cells</b>	3
<b>2. Cytokine Secretion Assays</b>	
The principle	4
Benefits	5
Applications and examples	6
<b>3. Intracellular cytokine staining</b>	
Antibodies	10
In-column staining	10
Intracellular Cytokine Detection Kits	11
Products for detection and enrichment of CD137 <sup>+</sup> and CD154 <sup>+</sup> cells	11
<b>4. Cell stimulation reagents</b>	
CytoStim	12
T Cell Activation / Expansion Kit	13
PepTivators: peptide pools for the specific stimulation of T cells	14
CMV pp65 – Recombinant Protein	14
<b>5. Related products</b>	15
<b>6. Product overview</b>	16

The cover illustration shows a section of a cytokine-secreting cell with the Golgi apparatus and secretory vesicles.

# MACS® Products for cytokine-producing cells

## Tools for the detection and enrichment of cytokine-producing cells

### Different kit configurations are available for various experimental needs

Miltenyi Biotec has developed valuable tools for the detection, enumeration, and isolation of cytokine-secreting leukocytes. The Cytokine-Secretion Assay – Detection Kits are designed for flow-cytometric detection of viable cytokine-secreting cells. Several detection kits with different fluorochromes are available and can be combined to study co-expression of cytokines or to counterstain peptide-MHC multimer-labeled cells.

The detection kits allow the analysis of cytokine-secreting cells at frequencies ranging from 0.01% to 0.1%. Enrichment of the cells prior to their analysis significantly increases the sensitivity of flow-cytometric detection: The Cytokine Secretion Assay – Cell Enrichment and Detection Kits enable the detection of cytokine-secreting cells at a frequency of one cell in a million.

Furthermore, the Cell Enrichment and Detection Kits allow the isolation of viable cytokine-secreting cells for subsequent experiments, for example, for cell culture, expansion, or for functional assays such as cytotoxicity assays.

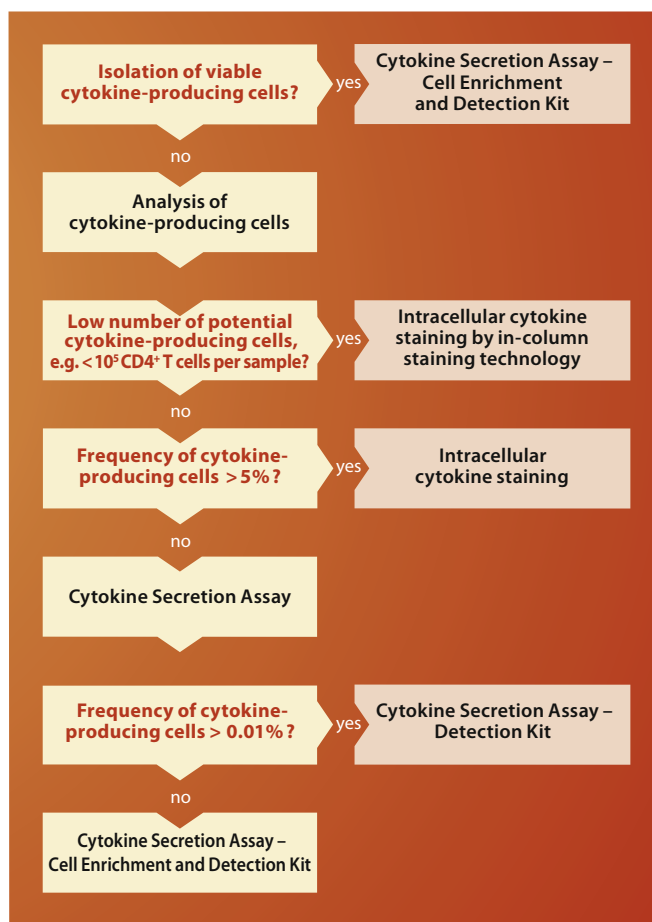
Additionally, a variety of antibodies and kits for intracellular cytokine staining is available.

## Cell stimulation reagents

Activated T cells are a major source of cytokines in immune responses. To facilitate studies on cytokine secretion by T cells, Miltenyi Biotec has developed a number of reagents and kits for the activation and expansion of these cells. Activation of T cells can be achieved by the antibody-based reagent CytoStim, which can be used as a positive control for cytokine expression or the expression of activation markers.

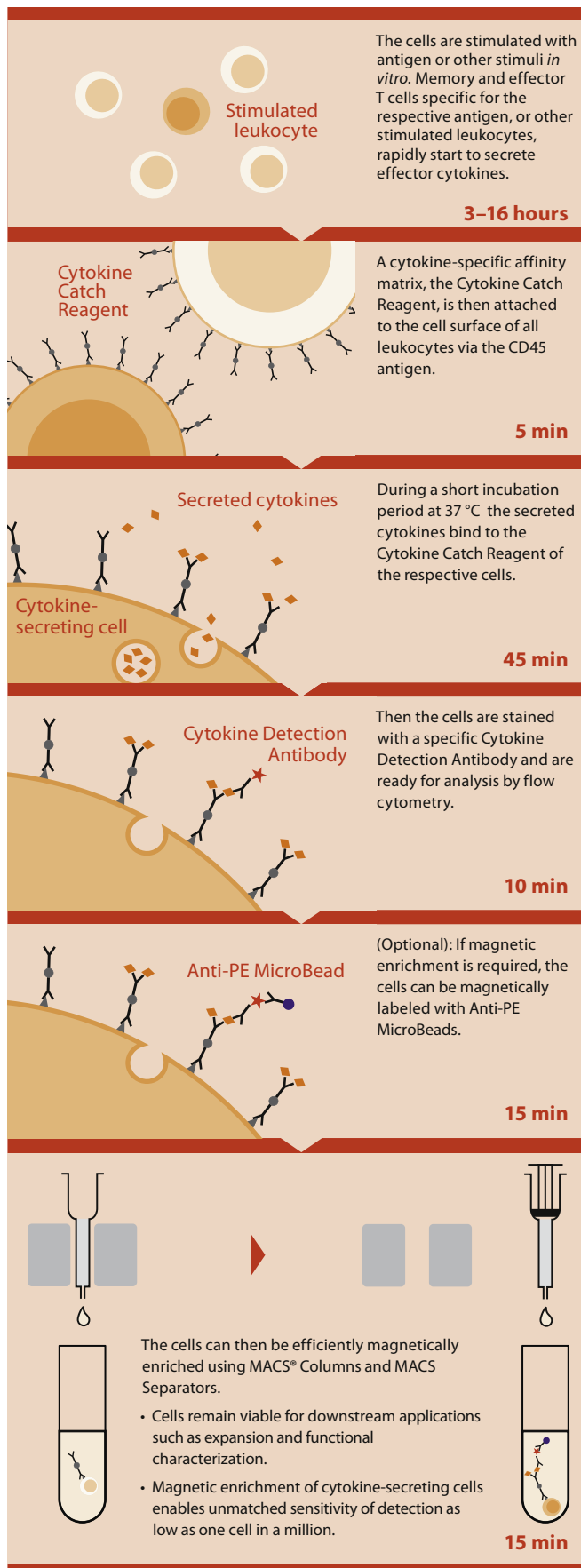
The T Cell Activation/Expansion Kit allows activation and, if desired, expansion of human T cells.

Antigen-specific T cells can be stimulated using recombinant proteins or the PepTivators—high-quality peptide pools for the stimulation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells.



Strategies for analysis and separation of cytokine-producing cells

# Cytokine Secretion Assays — the principle



## Analysis of cytokine-secreting cells

Cytokines are key mediators of immune responses. They are involved in inflammatory (IFN- $\gamma$ , TNF- $\alpha$ ) and humoral (IL-4, IL-5) immune reactions. Cytokines can promote growth and differentiation of cells in the immune system (IL-2), as well as suppression of immune reactions (IL-10).

The unique MACS® Cytokine Secretion Assay Technology enables analysis and enumeration of viable cytokine-secreting leukocytes by flow cytometry.

- For any cytokine-secreting CD45<sup>+</sup> leukocyte
- Analysis of cytokine secretion on single-cell level by flow cytometry
- Suitable for any antigen, e.g., proteins or peptides for T cell stimulation, glycolipids for NKT cell stimulation, LPS and other stimuli for monocytes
- Highly sensitive detection due to analysis of viable cells
- Sensitivity can be enhanced by magnetic enrichment to detect frequencies as low as one cytokine-secreting cell in a million
- Multiparametric analysis for phenotypic characterization

## Magnetic isolation of viable cytokine-secreting cells

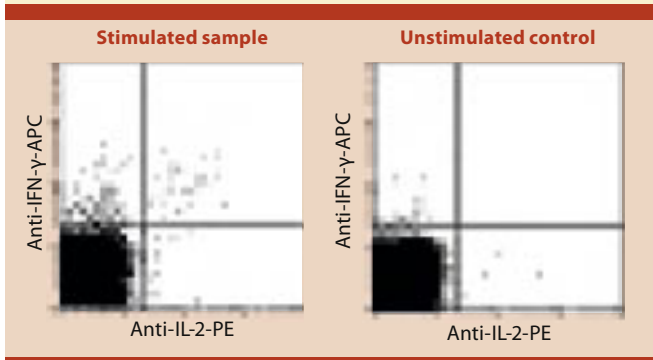
- MACS Cytokine Secretion Assay – Cell Enrichment and Detection Kits allow magnetic isolation of cytokine-secreting cells.
- Isolated cells remain viable and functional and can be further expanded.
- For various downstream applications, e.g., for functional characterization by molecular biology methods or cell culture.

# Benefits

## Provides more options than any other technology

- Detection of cytokine secretion at single-cell level
- Allows simultaneous multiparametric phenotyping of cytokine secreting cells to obtain information on subsets and activation status
- Allows detection of co-expression of two different cytokines
- Enables simultaneous staining of antigen-specific T cells with peptide-MHC tetramers, providing information on function of the cells
- For any leukocyte, for any antigen, for any human histocompatibility leukocyte antigen (HLA) type
- Optional magnetic enrichment of viable cytokine-secreting cells for enhanced sensitivity or downstream applications
- Detection of antigen-specific T cells and other leukocytes such as monocytes or dendritic cells (DCs) based on secretion of IFN- $\gamma$ , IL-2, IL-4, IL-5, IL-10, IL-12, or TNF- $\alpha$
- For human and murine cells

### Two-color cytokine analysis for detection of two different cytokines



**IFN- $\gamma$ - and IL-2-secreting CD4<sup>+</sup> T cells after stimulation with CMV lysate.** PBMCs of a CMV<sup>+</sup> donor were incubated for 16 hours with or without CMV lysate. The cells were stained for IFN- $\gamma$  and IL-2 secretion using the IFN- $\gamma$  Secretion Assay – Detection Kit (APC) in combination with the IL-2 Secretion Assay – Detection Kit (PE). The plots show data gated on CD4<sup>+</sup> cells.

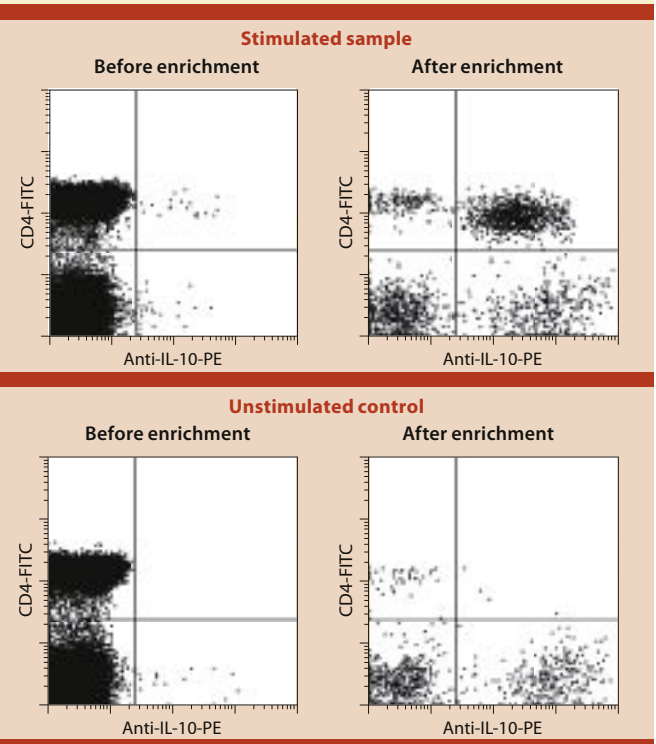
## Highly sensitive technology

- Analysis of viable cytokine-secreting cells increases sensitivity since dead cell exclusion minimizes non-specific background.
- Optional magnetic enrichment allows detection of one cytokine-secreting cell in a million (0.0001%).

## Fast and convenient procedure

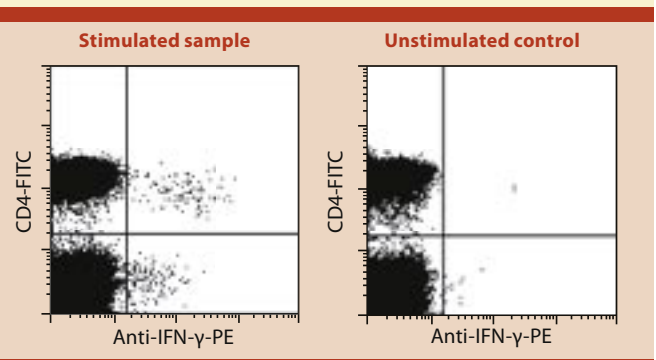
- Starting from whole blood, PBMCs, or other leukocyte-containing single-cell preparations
- Optimized easy-to-follow protocol
- Reliable results in less than two hours after cell stimulation

### Increased sensitivity of detection by prior cell enrichment



**IL-10-secreting CD4<sup>+</sup> T cells after stimulation with CMV lysate.** PBMCs of a CMV<sup>+</sup> donor were stimulated for 16 hours with or without CMV lysate. The responding cells were stained and isolated according to secretion of IL-10 using the IL-10 Secretion Assay – Cell Enrichment and Detection Kit. The plots show data gated on viable lymphocytes.

### Cytokine detection in whole blood samples



**IFN- $\gamma$ -secreting CD4<sup>+</sup> T cells after stimulation with EBV lysate.** 250  $\mu$ L of whole blood per sample of an EBV<sup>+</sup> donor was incubated for 4 hours with or without EBV lysate. The cells were stained for IFN- $\gamma$  secretion using the IFN- $\gamma$  Secretion Assay – Detection Kit (PE). The plots show data gated on viable lymphocytes.

# Applications and examples

The Cytokine Secretion Assay opens up new perspectives in immunology research by providing a technology for the detection, enumeration, and isolation of viable cytokine-producing leukocytes. The assay allows cytokine secretion analysis of any immunoreactive cell expressing CD45, e.g., antigen-specific T cells, NKT cells, NK cells, B cells, monocytes/macrophages, and DCs.

## Antigen-specific T cells

The Cytokine Secretion Assay was initially developed for sensitive detection and isolation of antigen-specific T cells. The analysis of their functionality can provide valuable information on the specificity of T cell immunity and on the progression of an immune response. This is relevant for research on vaccination and the development of treatment of various diseases involving infections and autoimmunity.

Moreover, the highly sensitive analysis of functionally active antigen-specific T cells is a prerequisite for the identification of antigens in disease, for TCR epitope mapping, or analysis of the TCR repertoire.

Antigen-specific T cell restimulation can be performed with specific peptides, proteins, glycolipids, or crude antigen preparations such as whole cells or cell extracts.

Please refer to the section on cell stimulation reagents for a variety of products. The antigen can be added directly to PBMCs, whole blood, or other single-cell preparations containing leukocytes. Examples for applications include:

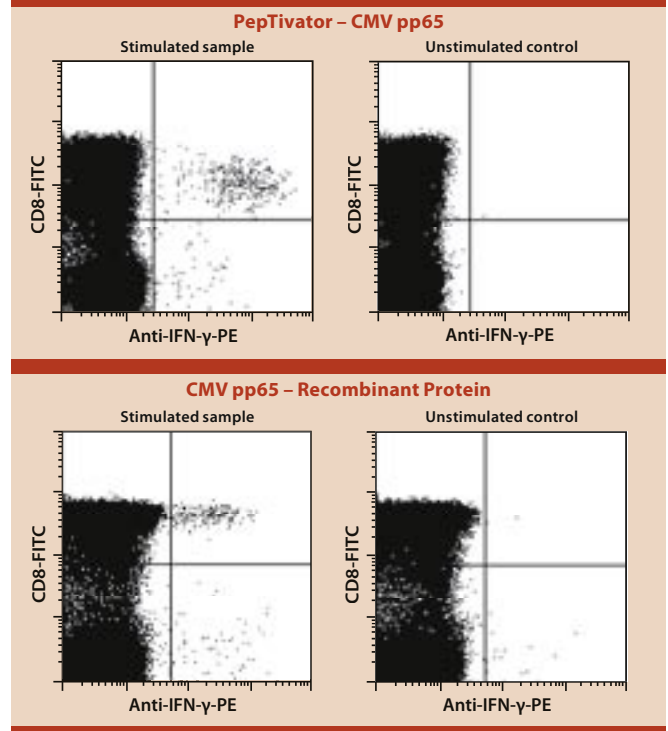
- TH1 and TH2 development and commitment<sup>1</sup>
- Highly sensitive detection of viable antigen-specific T cells for enumeration, phenotypic, and functional characterization<sup>2</sup>
- Analysis and enrichment of cytokine-secreting cells for identification of novel antigens and for T cell receptor epitope mapping<sup>3</sup>
- Quantification of antigen-specific T cells, e.g., CD4<sup>+</sup> memory T cells<sup>4</sup>
- Analysis of TCR repertoire of antigen-specific T cells<sup>5</sup>
- Analysis of cytokine expression by peptide-MHC multimer-stained cells<sup>6</sup>

### Special protocols

The following protocols are available at [www.miltenyibiotec.com/protocols](http://www.miltenyibiotec.com/protocols)

- Short protocols
- Cytokine Secretion Assays for whole blood
- Two-color Cytokine Secretion Assays
- Combined staining of cytokine-secreting cells with peptide-MHC multimers
- Expansion of antigen-specific cytokine-secreting T cells

### Stimulation with any antigen



**IFN-γ-secreting T cells after stimulation with CMV pp65 – Recombinant Protein or PepTivator – CMV pp65.** PBMCs of a CMV<sup>+</sup> donor were stimulated with CMV pp65 – Recombinant Protein or PepTivator – CMV pp65, or left untreated. All samples were stained for secreted IFN-γ using the IFN-γ Secretion Assay – Detection Kit (PE). T cells were counterstained for CD8 expression. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence. IFN-γ secretion of viable lymphocytes is shown.

## Simultaneous staining of cytokines and peptide-MHC multimers

The Cytokine Secretion Assays can be used in combination with peptide-MHC multimer staining to provide more information about the function of peptide-specific T cells.<sup>6,7</sup>

A special protocol is available at:

[www.miltenyibiotec.com/protocols](http://www.miltenyibiotec.com/protocols)

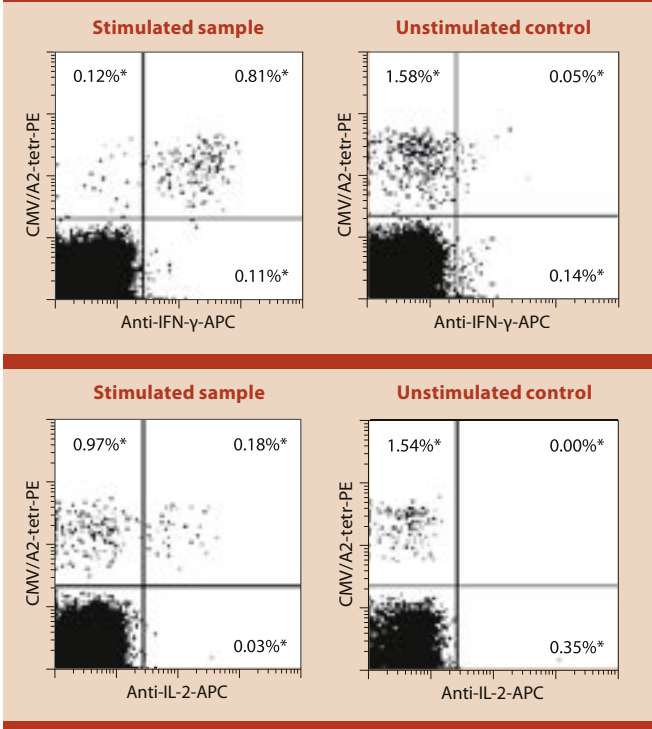
## Isolated cells remain viable

### and functional for downstream applications

The Cytokine Secretion Assays allow the isolation of viable cytokine-secreting cells.<sup>2,8,9</sup> In the example shown, isolated antigen-specific T cells have been expanded and functionally characterized, e.g., tested for antigen-specific cytotoxicity. A special protocol for expansion of cytokine-secreting antigen-specific T cells is available at:

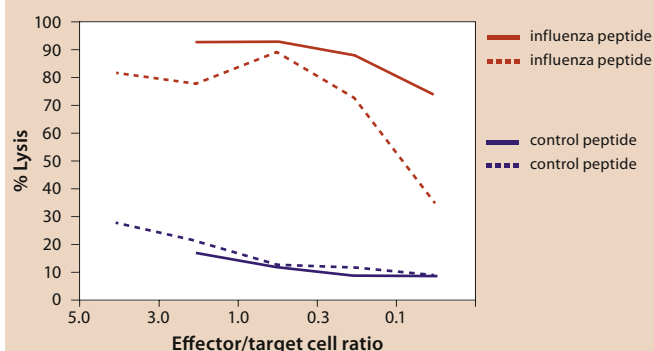
[www.miltenyibiotec.com/protocols](http://www.miltenyibiotec.com/protocols)

### Combination with peptide-MHC multimers



**IFN- $\gamma$ - or IL-2-secreting cells costained with CMV/A2-tetramers.** PBMCs of a HLA/A2 CMV<sup>+</sup> donor were labeled with PE-conjugated CMVpp65/HLA/A2-tetramers and then incubated with or without CMV peptide pp65<sub>495-503</sub>. The samples were stained for cytokine secretion using the IFN- $\gamma$  Secretion Assay – Detection Kit (APC) or the IL-2 Secretion Assay – Detection Kit (APC). The plots show data gated on viable CD8<sup>+</sup> T cells.

### Isolation of viable and functional cells



### Isolated IFN- $\gamma$ -secreting CD8<sup>+</sup> T cells show specific lysis of target cells.

Influenza peptide-specific CD8<sup>+</sup> T cells were enriched using the IFN- $\gamma$  Secretion Assay – Cell Enrichment and Detection Kit (PE) and expanded for two weeks in the presence of IL-2. The cells were then tested for cytotoxicity against a target cell line loaded with influenza peptide or with a control peptide. Highly specific lysis was observed even at low effector-to-target cell ratios. Results from two different experiments are shown.

## References

- Ouyang, W. *et al.* (2000) Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. *Immunity* 12: 27–37.
- Desombere, I. *et al.* (2004) The interferon gamma secretion assay: a reliable tool to study interferon gamma production at the single-cell level. *J. Immunol. Methods* 286: 167–185.
- Bitmansour, A. D. *et al.* (2002) Direct *ex vivo* analysis of human CD4<sup>+</sup> memory T cell activation requirements at the single clonotype level. *J. Immunol.* 169: 1207–1218. [2675]
- Sojka, D. K. *et al.* (2004) IL-2 secretion by CD4<sup>+</sup> T cells *in vivo* is rapid, transient, and influenced by TCR-specific competition. *J. Immunol.* 172: 6136–6143.
- Cohen, G. B. *et al.* (2002) Clonotype tracking of TCR repertoires during chronic virus infections. *Virology* 304: 474–484.
- Meidenbauer, N. *et al.* (2003) Survival and tumor localization of adoptively transferred melan-A-specific T cells in melanoma patients. *J. Immunol.* 170: 2161–2169.
- Pittet, M. J. *et al.* (2001) *Ex vivo* IFN- $\gamma$  secretion by circulating CD8 T lymphocytes: implications of a novel approach for T cell monitoring in infectious and malignant diseases. *J. Immunol.* 166: 7634–7640.
- Brostherus, H. *et al.* (1999) Enrichment and detection of live antigen-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells based on cytokine secretion. *Eur. J. Immunol.* 29: 4053–4059.
- Rauser, G. *et al.* (2004) Rapid generation of combined CMV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell lines for adoptive transfer into recipients of allogeneic stem cell transplants. *Blood* 103: 3565–3572.

# Applications and examples

## Regulatory T cells

Different types of regulatory T cells have gained attention over the last years by researchers in basic and clinical immunology. Besides the CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells (Tregs), Type 1 regulatory T cells (Tr cells) are of great interest, e.g., in research on autoimmune diseases. Tr cells are characterized by their capacity to produce large amounts of IL-10 and TGF- $\beta$ . Key cytokines for the analysis of suppressor function are IL-2 and IL-10. The latter is considered to be one of the main molecules involved in immunosuppression; a target for suppression seems to be the transcriptional control of IL-2 in effector cells.

The Cytokine Secretion Assay technology represents a unique tool for the analysis of IL-2 and IL-10 production by Tregs and suppressed T cells.

The IL-2 Secretion Assay has been used for single-cell kinetic analyses of CD4<sup>+</sup>CD25<sup>+</sup> T cell-mediated suppression<sup>1</sup> and for determination of the frequency of IL-2-producing responder cells in the presence of regulatory T cells<sup>2</sup>.

The IL-10 Secretion Assay has been used for the enumeration of type 1 regulatory T cells in healthy individuals in comparison to patients with an autoimmune skin disease.<sup>3</sup>

The IL-4 and IL-10 Secretion Assays have been used to analyze allergen-specific Tr cells and T<sub>H</sub>2 cells.<sup>4</sup>

### References

1. Sojka, D. K. *et al.* (2005) Early kinetic window of target T cell susceptibility to CD25 regulatory T cell activity. *J. Immunol.* 175: 7274–7280.
2. de la Rosa, M. *et al.* (2004) Interleukin-2 is essential for CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cell function. *Eur. J. Immunol.* 34: 2480–2488.
3. Veldman, C. *et al.* (2004) Type I regulatory T cells specific for desmoglein 3 are more frequently detected in healthy individuals than in patients with pemphigus vulgaris. *J. Immunol.* 172: 6468–6475.
4. Akdis, M. *et al.* (2004) Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J. Exp. Med.* 199: 1567–1575.

## NK cells

NK cells represent a distinct lymphocyte population with potent cytolytic activity and a variety of other effector functions. They are considered non-MHC-restricted cytotoxic cells and produce type 1 cytokines such as IFN- $\gamma$  and TNF- $\alpha$ .

The IFN- $\gamma$  Secretion Assay has been used to separate two NK cell subsets according to IFN- $\gamma$  secretion. Moreover, the isolated NK cell subsets were expanded and their cytokine profile examined, demonstrating viability and functionality of the isolated cells.<sup>1</sup> Furthermore, the effects of IL-4 on the production of IFN- $\gamma$  by NK and NKT cells was investigated.<sup>2</sup>

### References

1. Deniz, G. *et al.* (2002) Human NK1 and NK2 subsets determined by purification of IFN- $\gamma$ -secreting and IFN- $\gamma$ -non-secreting NK cells. *Eur. J. Immunol.* 32: 879–884.
2. Morris, S. C. *et al.* (2006) IL-4 induces *in vivo* production of IFN- $\gamma$  by NK and NKT cells. *J. Immunol.* 176: 5299–5305.

## NKT cells

NKT cells are a population of T cells that share some characteristics with natural killer (NK) cells and exert important regulatory functions. An important role for NKT cells might be to protect self-tissues from deleterious inflammatory-type immune responses. In addition, there is evidence that they can control immune responses to infections and some tumors.

However, the population of NKT cells is very heterogeneous and a precise definition of this cell type still remains elusive.

The Cytokine Secretion Assay has been utilized for the analysis of IL-2, IL-4, and IFN- $\gamma$  production of NKT cells in a systemic lupus erythematosus mouse model.<sup>1</sup>

Furthermore, the cytokine profiles of NKT cells from healthy subjects and NKT cells from multiple sclerosis patients in remission have been compared using the IFN- $\gamma$  and IL-4 Secretion Assay – Detection Kits.<sup>2</sup>

### References

1. Yang, J. Q. *et al.* (2003) Immunoregulatory role of CD1d in the hydrocarbon oil-induced model of lupus nephritis. *J. Immunol.* 171: 2142–2153.
2. Araki, M. *et al.* (2003) Th2 bias of CD4<sup>+</sup> NKT cells derived from multiple sclerosis in remission. *Int. Immunol.* 15: 279–288.

## B cells

The outcome of interactions between B cells and T cells is highly regulated by cytokines. Cytokines secreted by T cells are considered to play a predominant role in this interplay since these cytokines are able to influence B cell differentiation. However, activated B cells also can produce significant amounts of cytokines. Indeed, there is growing interest in the potential of B cells to modulate immune responses.

B cell responses and cytokine profiles have been analyzed following activation mediated by the B cell receptor (BCR) or via CD40 engagement.

Activation through both the BCR and CD40 leads to production of, e.g., IL-6 and TNF- $\alpha$ , whereas B cells activated through CD40 without preceding antigen/BCR engagement produce significant amounts of the immunoregulatory cytokine IL-10. IL-10 secretion by B cells has also been studied using the Mouse IL-10 Secretion Assay.<sup>1</sup> IL-10 secretion was analyzed after *in vitro* stimulation of neonatal spleen cells with CpG oligodeoxynucleotides. Cells were costained with B cell phenotype markers. In this experimental setting, the CD45R (B220)<sup>+</sup> and CD19<sup>+</sup> B cells, but not CD3<sup>+</sup> T cells or CD11b<sup>+</sup> myeloid cells, were responsible for the main IL-10 production.

### Reference

1. Sun, C.-M. *et al.* (2005) Upon TLR9 signaling, CD5<sup>+</sup> B cells control the IL-12-dependent Th1-priming capacity of neonatal DCs. *Immunity* 22: 467–477.

## Dendritic cells

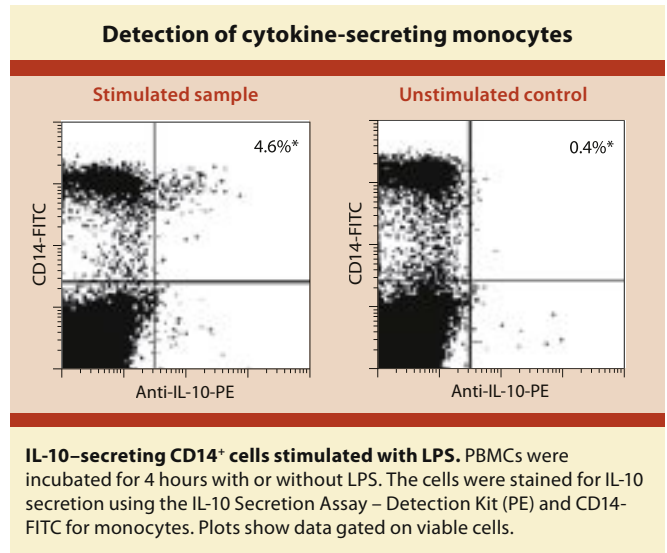
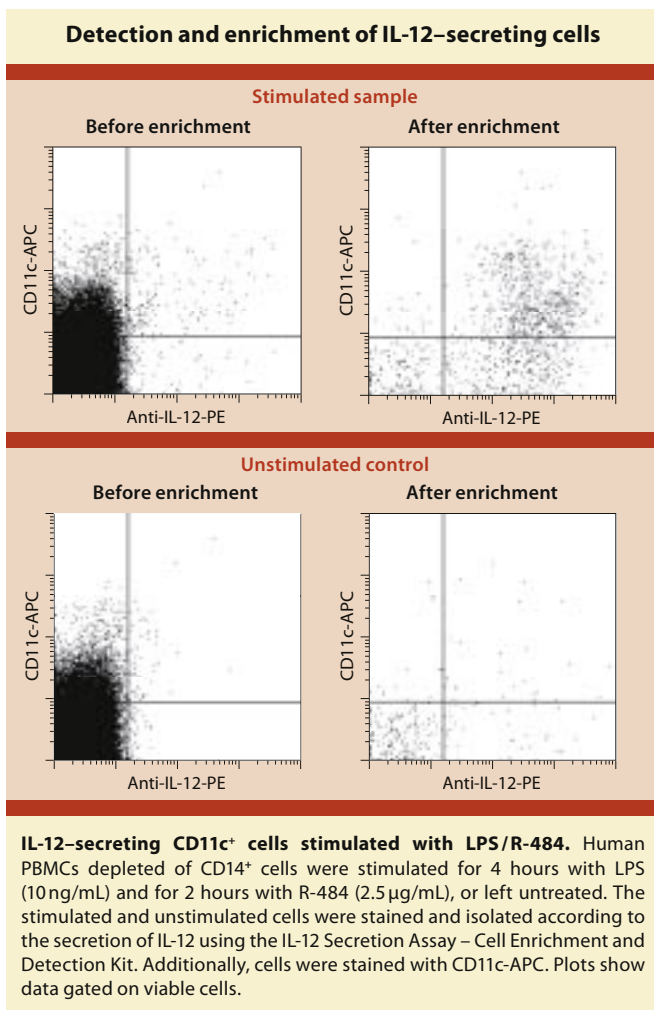
Dendritic cells (DCs) play an important role in both the priming of adaptive immune responses and the induction of self-tolerance. In humans as well as in mice, DCs represent a heterogeneous cell population comprising DC subsets distinct with regard to their phenotypes and functions. DCs are potent antigen-presenting cells that provide costimulation for T cells, thus being most effective in initiating primary T cell responses. The Cytokine Secretion Assay technology has been used for the analysis of the stimulatory capacity of DCs, e.g., by analyzing the T cell response to stimulation with mature or immature DCs<sup>1</sup>, or with CD40L-transfected DCs<sup>2</sup>. Direct analysis of cytokine production by DCs after infection with mycobacteria was investigated using the IFN- $\gamma$  Secretion Assay - Detection Kit.<sup>3</sup> The capability of CD8 $\alpha^+$  DCs to secrete IL-10 after stimulation with microbial stimuli and CD40L was demonstrated in a mouse

## References

1. Ponsaerts, P. *et al.* (2002) Messenger RNA electroporation of human monocytes, followed by rapid *in vitro* differentiation, leads to highly stimulatory antigen-loaded mature dendritic cells. *J. Immunol.* 169: 1669–1675.
2. Dannull, J. *et al.* (2005) Enhancing the immunostimulatory function of dendritic cells by transfection with mRNA encoding OX40 ligand. *Blood* 105: 3206–3213.
3. Fricke, I. *et al.* (2006) Mycobacteria induce IFN- $\gamma$  production in human dendritic cells via triggering of TLR2. *J. Immunol.* 176: 5173–5182.
4. Edwards, A. D. *et al.* (2002) Microbial recognition via Toll-like receptor-dependent and -independent pathways determines the cytokine response of murine dendritic cell subsets to CD40 triggering. *J. Immunol.* 169: 3652–3660.

## Monocytes

Monocytes are essential effector cells in chronic inflammatory diseases and also in fighting infections. To exert their functions, monocytes need to be activated, either via inflammatory cytokines produced by the adaptive immune system or via direct stimulation by bacterial products. Monocytes are activated through ligation of pattern-recognition receptors such as Toll-like receptors (TLRs) by microbial ligands (e.g. LPS, CpG DNA) and are capable of secreting cytokines such as IL-10, TNF- $\alpha$ , or IL-12, depending on the stimulus.



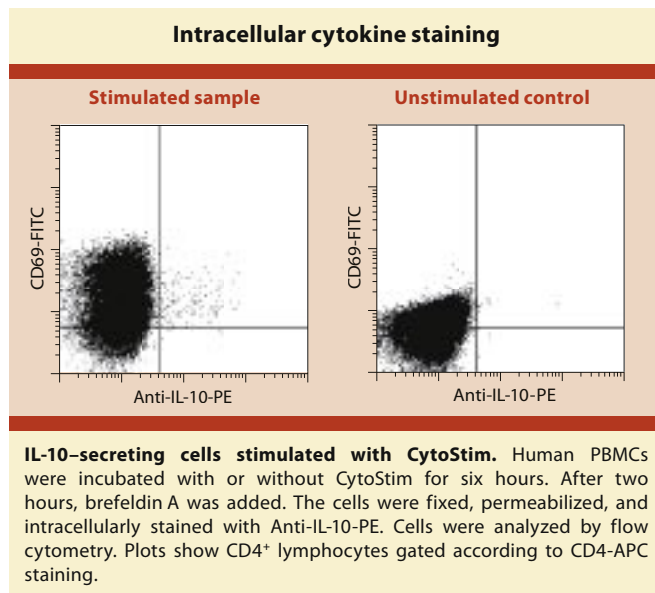
# Intracellular cytokine staining

## Antibodies for intracellular cytokine staining

Intracellular fluorescent staining of cytokines in immunoreactive cells is a widely used technique for monitoring immune responses. Miltenyi Biotec offers a number of anti-cytokine antibodies for the intracellular staining of cytokines.

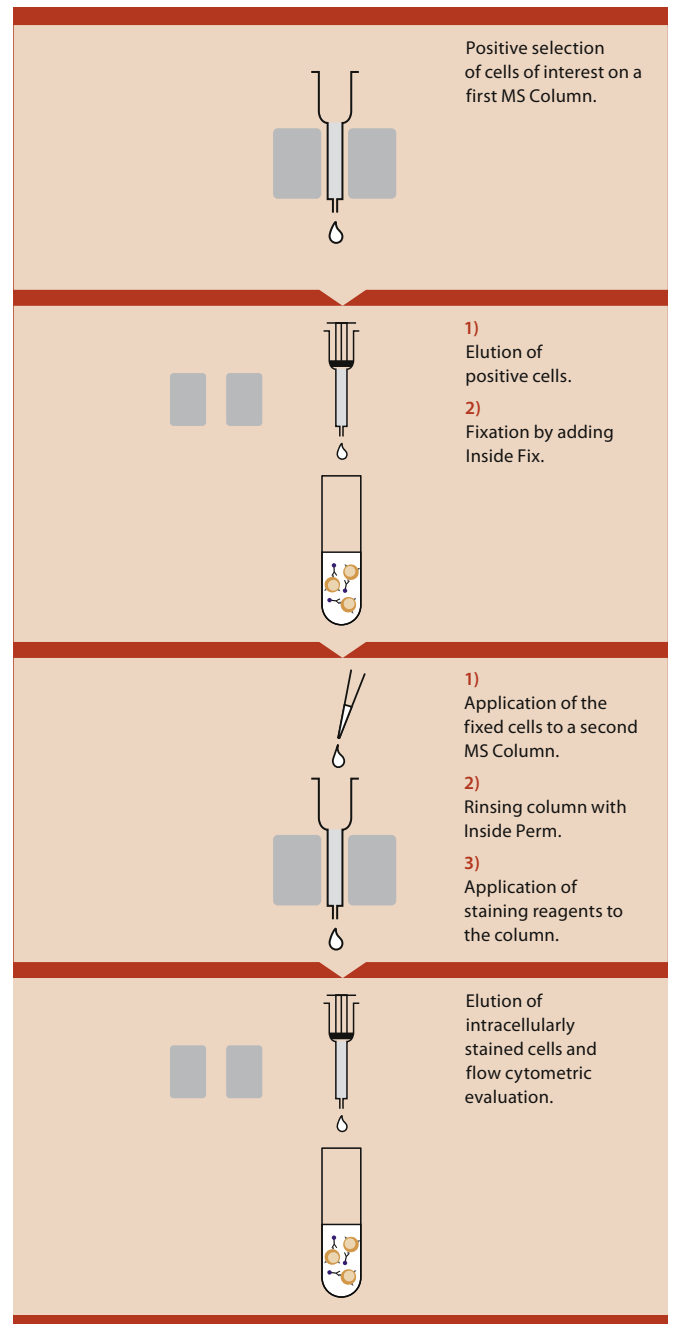
After stimulation of the cells in the presence of a secretion inhibitor, the cells are fixed and permeabilized using the Inside Stain Kit. Subsequently, cells are stained intracellularly with anti-cytokine antibodies. The procedure can either be performed using cell suspensions or by in-column intracellular staining in combination with MACS® Technology. This innovative protocol allows intracellular cytokine staining in combination with MACS Separation of the cells of interest. After the cells have been magnetically labeled and fixed, they are immobilized on a MACS Column placed in a MACS Separator for the permeabilization and staining procedure.

This technology allows fast, efficient, and gentle intracellular cytokine staining with minimal loss of cells, since time-consuming centrifugation steps during cell washes are avoided. Furthermore, cells of interest, e.g., certain subsets such as CD4<sup>+</sup> or CD8<sup>+</sup> cells, can be pre-selected by magnetic enrichment for a more sensitive and accurate flow cytometric analysis. The in-column intracellular staining technology is especially useful when working with samples that contain a low number of cells of interest, or with cells other than PBMCs, e.g., broncho-alveolar lavages or synovial fluids.



## In-column intracellular cytokine staining

- Gentle staining procedure
- Minimal loss of cells
- Enrichment of target cells for higher sensitivity
- Faster than conventional staining protocols



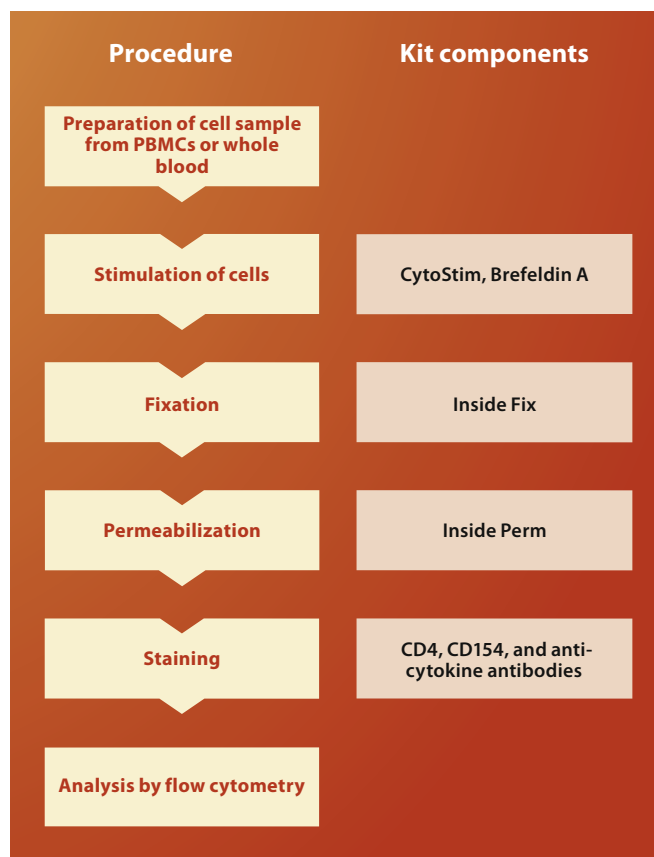
In-column intracellular staining of cells isolated by MACS® Technology.

## Intracellular Cytokine Detection Kits

Miltenyi Biotec has developed a panel of Cytokine Detection Kits for the rapid and reliable flow cytometric detection of cytokine production in activated human CD4<sup>+</sup> T cells. CD154 is up-regulated in CD4<sup>+</sup> T cells within hours after activation and is used as a specific marker for antigen-specific T cell activation. By combining detection of cytokine production with staining of CD154 and CD4, the sensitive and specific detection of activated antigen-specific CD4<sup>+</sup> T cells can be performed starting from human whole blood or PBMCs. Cytokine Detection Kits are available for the detection of IFN- $\gamma$ , TNF- $\alpha$ , IL-2, IL-4, or IL-5. Each kit contains the reagents for permeabilization, fixation, and immunofluorescent staining of cytokine-producing CD4<sup>+</sup> T cells, as well as CytoStim for *in vitro* stimulation of CD4<sup>+</sup> T cells providing a reliable positive control and Brefeldin A.

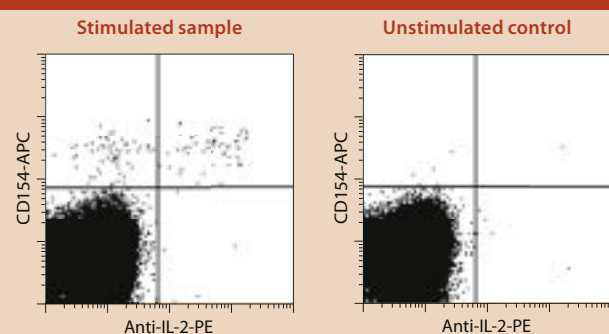
The simple and fast procedure allows:

- Flow cytometric identification and enumeration of cytokine-producing, CD4<sup>+</sup>CD154<sup>+</sup> T cells upon *in vitro* stimulation or restimulation with the respective antigen
- Monitoring the antigen specificity of T cell clones
- Identification and monitoring of *in vivo* T cell responses in whole blood samples or PBMCs



Complete kits for intracellular cytokine staining: procedure

### Cytokine staining in activated antigen-specific CD4<sup>+</sup> T cells



**IL-2-secreting CD4<sup>+</sup> T cells activated with CMV pp65 – Recombinant Protein.** Human PBMCs were incubated with or without CMV pp65 – Recombinant Protein for 6 hours. After 2 hours, brefeldin A was added. Cells were fixed, permeabilized, and stained with CD154-APC, Anti-IL-2-PE, and CD4-FITC using the CD154/IL-2/CD4 Detection Kit. Cells were analyzed by flow cytometry. Plots show data gated on viable CD4<sup>+</sup> cells.

## Products for detection and enrichment of CD137<sup>+</sup> and CD154<sup>+</sup> cells

The CD154 antigen plays an important role as a costimulatory molecule in the interaction between T cells and antigen-presenting cells through ligation of CD40. Due to its transient expression within hours after activation, CD154 represents a more reliable marker for activated antigen-specific CD4<sup>+</sup> T cells than traditionally used markers such as CD25 and CD69. Recently, also CD137 has been described to be a suitable marker for antigen-specific activation of T cells. Therefore, Miltenyi Biotec has developed several reagents for the detection and isolation of CD137<sup>+</sup> and CD154<sup>+</sup> activated T cells:

- CD137 MicroBead Kit, human
- CD137 antibodies, human
- CD154 antibodies, human and mouse
- CD154 MicroBead Kit, human
- CD154 Enrichment and Detection Kit, human
- CD154 Detection Cocktails, mouse

# Cell stimulation reagents

Activated T cells produce large amounts of various cytokines in immune responses. To complement the comprehensive product portfolio for the detection of cytokines, Miltenyi Biotec has developed a number of tools for T cell activation:

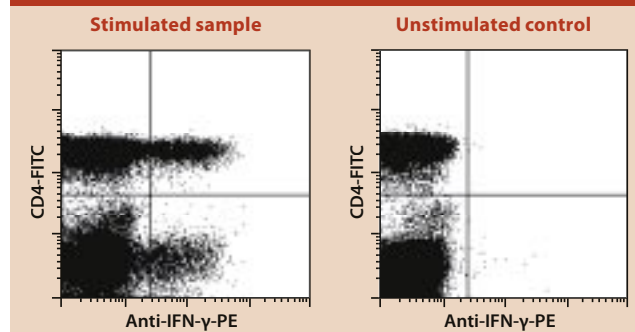
- CytoStim for rapid and efficient T cell stimulation
- A complete kit for the activation and expansion of T cells
- Antigens for the specific stimulation of T cells: recombinant proteins, and peptide pools.

## CytoStim

CytoStim has been developed for the rapid and efficient restimulation of human effector/memory T cells. CytoStim is an antibody-based reagent that acts in a similar fashion as a superantigen but independently of certain V $\beta$  domains of the T cell receptor (TCR). It causes activation of T cells by cross-linking TCRs to MHC molecules of antigen-presenting cells. Upon stimulation with CytoStim, CD4<sup>+</sup> and CD8<sup>+</sup> T cells start to secrete cytokines or up-regulate activation markers on their cell surface within a few hours.

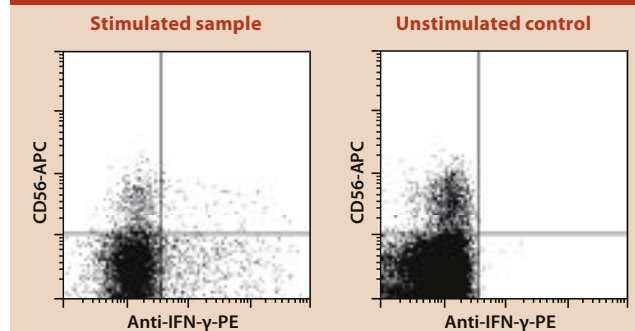
CytoStim is suitable for rapid stimulation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in fresh PBMCs, whole blood, or other leukocyte-containing single-cell suspensions from tissues. It can be used as a positive control in antigen-specific T cell stimulation assays or intracellular cytokine staining experiments to detect expression of cytokines or activation markers.

### Stimulation of T cells using CytoStim



**IFN- $\gamma$ -secreting CD4<sup>+</sup> T cells stimulated with CytoStim.** Human PBMCs were stimulated with CytoStim for 1 hour and stained according to their secretion of IFN- $\gamma$  using the IFN- $\gamma$  Secretion Assay – Detection Kit. T cells were counterstained with CD4-FITC. Plots show data gated on viable cells.

### Stimulation of NKT cells using CytoStim



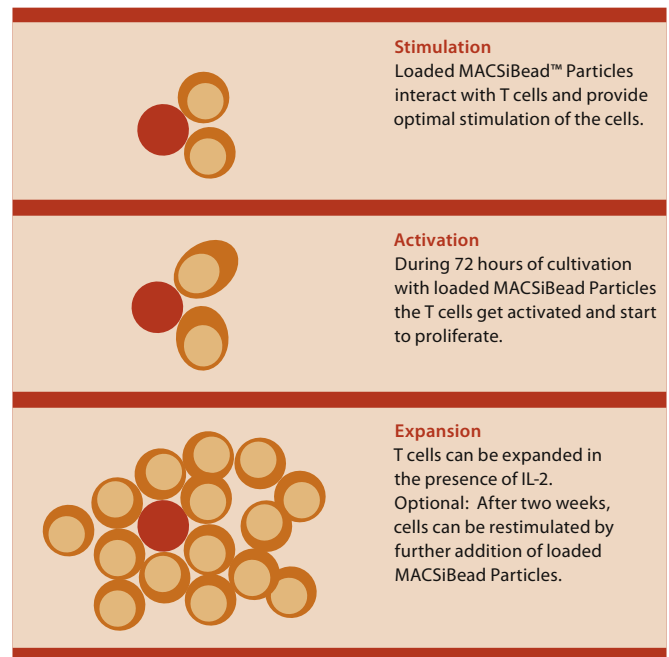
**IFN- $\gamma$ -secreting CD56<sup>+</sup> T cells stimulated with CytoStim.** Human PBMCs were stimulated with CytoStim and intracellularly stained with Anti-IFN- $\gamma$ -PE. NKT cells were counterstained with CD3-FITC and CD56-APC. The plots are gated on CD3<sup>+</sup> cells.

## T Cell Activation/Expansion Kits

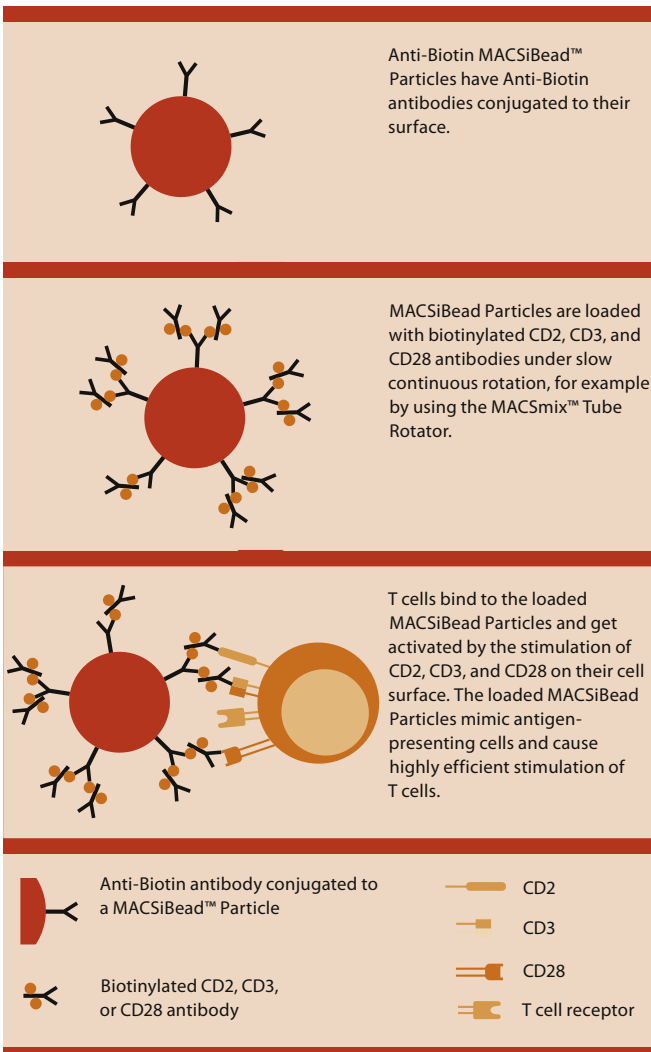
Unlike magnetic cell separation with MACS® MicroBeads, separation with larger magnetic particles tends to result in cell activation. Taking advantage of this phenomenon, Miltenyi Biotec has developed large micron-sized magnetic particles—MACSiBead™ Particles for cell activation.

The T Cell Activation/Expansion Kit is designed for the activation and, if required, the expansion of human T cells. The kit consists of Anti-Biotin MACSiBead Particles and biotinylated antibodies against human CD2, CD3, and CD28. Anti-Biotin MACSiBead Particles, loaded with biotinylated antibodies, are used to mimic antigen-presenting cells and activate resting T cells from PBMCs as well as purified T cells or Jurkat cells. T cell expansion is achieved by culturing and reactivation at day 14 of culture.

T Cell Activation and Expansion Kits are available for human and non-human primate cells.



Activation and expansion of T cells with loaded MACSiBead™ Particles.



Loading of MACSiBead™ Particles for stimulation of T cells.

# Cell stimulation reagents

## PepTivators: peptide pools for the specific stimulation of T cells

The new PepTivators—high-quality peptide pools—consist of 15-mer peptides with 11-amino acid (aa) overlap covering the complete sequence of the respective antigen, for example, the CMV proteins pp65 and IE-1. PepTivators are designed for efficient *in vitro* stimulation of antigen-specific CD4<sup>+</sup> or CD8<sup>+</sup> T cells, as peptides of 15 aa length with 11-aa overlap represent an optimized solution for stimulating both CD4<sup>+</sup> and CD8<sup>+</sup> T cells in various applications.

Two formats of PepTivators allow the stimulation of 10<sup>8</sup> cells (6 nmol/peptide) or 10<sup>9</sup> cells (60 nmol/peptide). Available peptide pools include:

- PepTivator – CMV pp65
- PepTivator – CMV IE-1
- PepTivator – AdV5 Hexon

For the most recent additions to our peptide pool portfolio, please visit [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

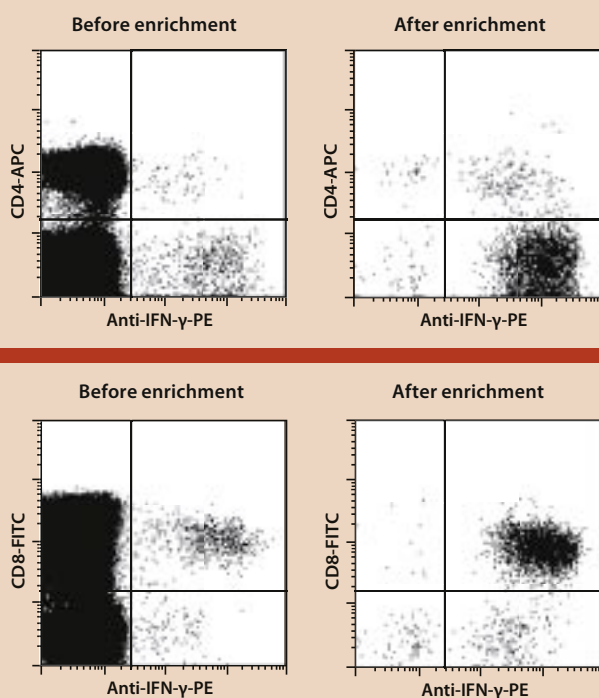
## CMV pp65 – Recombinant Protein

CMV pp65 – Recombinant Protein is designed for restimulation of antigen-specific T cells in order to detect and analyze human pp65-specific CD4<sup>+</sup> and CD8<sup>+</sup> effector/memory T cells by MACS<sup>®</sup> Cytokine Secretion Assays, intracellular cytokine staining, or other techniques.

Further applications for the restimulation of cells using CMV pp65 – Recombinant Protein include:

- Isolation of viable pp65-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells, using MACS Cytokine Secretion Assay – Cell Enrichment and Detection Kits.
- Generation of pp65-specific CD4<sup>+</sup> and CD8<sup>+</sup> effector/memory T cells from naive T cell populations, e.g., for research on immunotherapy and vaccination.
- Pulsing of antigen-presenting cells, e.g., for research on dendritic cell vaccination as well as for research on antigen delivery by antigen-presenting cells.

### Stimulation of antigen-specific T cells using PepTivator – CMV pp65



**Detection and isolation of viable pp65-specific T cells using PepTivator – CMV pp65 and the IFN-γ Secretion Assay.** PBMCs of a CMV<sup>+</sup> donor were restimulated for 4 hours with 20 μL/mL of reconstituted PepTivator – CMV pp65. T cells specific for pp65 were stained and magnetically enriched according to their secretion of IFN-γ using the IFN-γ Secretion Assay – Cell Enrichment and Detection Kit. T cells were counterstained for CD4 and CD8 expression. IFN-γ secretion of viable lymphocytes is shown.

### Stimulation of antigen-specific T cells using CMV pp65 – Recombinant Protein



**Detection and isolation of viable pp65-specific T cells using CMV pp65 – Recombinant Protein and the CD154 MicroBead Kit.** PBMCs were stimulated for 16 hours with CMV pp65 – Recombinant Protein (# 130-091-823), and a CD40 blocking-antibody was added during the stimulation to prevent down-regulation of CD154. Subsequently, CD154<sup>+</sup> cells were separated using the CD154 MicroBead Kit. Cell fractions are fluorescently stained with Anti-Biotin-PE and CD4-FITC. Plots show data gated on viable cells.

# Related products

## MACSmix™ Tube Rotator

The accuracy of the Cytokine Secretion Assays crucially depends on a continuous resuspension of the cells during the secretion period to avoid cross-capture of cytokines. The MACSmix™ Tube Rotator has been developed to provide optimal incubation conditions for Cytokine Secretion Assays and any application where slow rotation of sample tubes is needed.

The device runs on rechargeable batteries and operates independently of a permanent power supply. It is suitable for a temperature range of 2 °C to 42 °C and can be placed in a refrigerator or incubator.

The versatile MACSmix Tube Rotator can be operated with two different racks designed for use with tubes from 0.5 mL to 50 mL in capacity. The instrument operates at three different speeds or at pre-set intervals.



## MACS® Antibodies

MACS® Antibodies are optimized for the evaluation of MACS Cell Separations (MACS Control) allowing staining of cells during or after magnetic cell isolation. For the full range of antibodies, please visit [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

## MACS Cytokines and Growth Factors

Miltenyi Biotec now offers a broad portfolio of more than 150 cytokines, growth factors, and related proteins. These high-quality products are well-suited for various applications such as cell culture, differentiation studies, and biological assays. Selected products are available in a premium-grade quality with high, well-defined activity as well as in research-grade quality. For the full range of cytokines and growth factors, please visit [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

# Product overview

## Cytokine Secretion Assays – Cell Enrichment and Detection Kits

Product	Capacity	Order no.
<b>Human cells</b>		
Large Scale IFN- $\gamma$ Secretion Assay – Enrichment Kit	10 <sup>9</sup> total cells	130-091-329
IFN- $\gamma$ Secretion Assay – Cell Enrichment and Detection Kit (PE)	50 tests with 10 <sup>7</sup> total cells	130-054-201
IL-2 Secretion Assay – Cell Enrichment and Detection Kit (PE)	50 tests with 10 <sup>7</sup> total cells	130-090-488
IL-4 Secretion Assay – Cell Enrichment and Detection Kit (PE)	50 tests with 10 <sup>7</sup> total cells	130-054-101
IL-5 Secretion Assay – Cell Enrichment and Detection Kit (PE)	50 tests with 10 <sup>7</sup> total cells	130-091-622
IL-10 Secretion Assay – Cell Enrichment and Detection Kit (PE)	50 tests with 10 <sup>7</sup> total cells	130-090-435
IL-12 Secretion Assay – Cell Enrichment and Detection Kit (PE)	50 tests with 10 <sup>7</sup> total cells	130-092-122
IL-13 Secretion Assay – Cell Enrichment and Detection Kit (PE)	50 tests with 10 <sup>7</sup> total cells	130-093-480
TNF- $\alpha$ Secretion Assay – Cell Enrichment and Detection Kit (PE)	50 tests with 10 <sup>7</sup> total cells	130-091-269
IFN- $\alpha$ Secretion Assay – Cell Enrichment and Detection Kit (PE)	50 tests with 10 <sup>7</sup> total cells	<i>coming soon</i>
<b>Mouse cells</b>		
Mouse IFN- $\gamma$ Secretion Assay – Cell Enrichment and Detection Kit (PE)	50 tests with 10 <sup>7</sup> total cells	130-090-517
Mouse IL-2 Secretion Assay – Cell Enrichment and Detection Kit (PE)	50 tests with 10 <sup>7</sup> total cells	130-090-492
Mouse IL-4 Secretion Assay – Cell Enrichment and Detection Kit (PE)	50 tests with 10 <sup>7</sup> total cells	130-090-515
Mouse IL-5 Secretion Assay – Cell Enrichment and Detection Kit (PE)	50 tests with 10 <sup>7</sup> total cells	130-091-175
Mouse IL-10 Secretion Assay – Cell Enrichment and Detection Kit (PE)	50 tests with 10 <sup>7</sup> total cells	130-090-490
Mouse IL-17 Secretion Assay – Cell Enrichment and Detection Kit (PE)	50 tests with 10 <sup>7</sup> total cells	<i>coming soon</i>

## Cytokine Secretion Assays – Detection Kits

Product	Conjugate	Capacity	Order no.
<b>Human cells</b>			
IFN- $\gamma$ Secretion Assay – Detection Kit	FITC	100 tests with 10 <sup>6</sup> cells	130-090-433
	PE	100 tests with 10 <sup>6</sup> cells	130-054-202
	APC	100 tests with 10 <sup>6</sup> cells	130-090-762
IL-2 Secretion Assay – Detection Kit	PE	100 tests with 10 <sup>6</sup> cells	130-090-487
	APC	100 tests with 10 <sup>6</sup> cells	130-090-763
IL-4 Secretion Assay – Detection Kit	PE	100 tests with 10 <sup>6</sup> cells	130-054-102
IL-5 Secretion Assay – Detection Kit	PE	100 tests with 10 <sup>6</sup> cells	130-091-623
	APC	100 tests with 10 <sup>6</sup> cells	130-091-624
IL-10 Secretion Assay – Detection Kit	PE	100 tests with 10 <sup>6</sup> cells	130-090-434
	APC	100 tests with 10 <sup>6</sup> cells	130-090-761

# Product overview

## Human cells

IL-12 Secretion Assay – Detection Kit	PE	100 tests with 10 <sup>6</sup> cells	130-092-124
IL-13 Secretion Assay – Detection Kit	PE	100 tests with 10 <sup>6</sup> cells	130-093-479
TNF-α Secretion Assay – Detection Kit	PE	100 tests with 10 <sup>6</sup> cells	130-091-268
	APC	100 tests with 10 <sup>6</sup> cells	130-091-267
IFN-α Secretion Assay – Detection Kit	PE	100 tests with 10 <sup>6</sup> cells	<i>coming soon</i>

## Mouse cells

Mouse IFN-γ Secretion Assay – Detection Kit	PE	100 tests with 10 <sup>6</sup> cells	130-090-516
	APC	100 tests with 10 <sup>6</sup> cells	130-090-984
Mouse IL-2 Secretion Assay – Detection Kit	PE	100 tests with 10 <sup>6</sup> cells	130-090-491
	APC	100 tests with 10 <sup>6</sup> cells	130-090-987
Mouse IL-4 Secretion Assay – Detection Kit	PE	100 tests with 10 <sup>6</sup> cells	130-090-479
Mouse IL-5 Secretion Assay – Detection Kit	PE	100 tests with 10 <sup>6</sup> cells	130-091-166
	APC	100 tests with 10 <sup>6</sup> cells	130-091-174
Mouse IL-10 Secretion Assay – Detection Kit	PE	100 tests with 10 <sup>6</sup> cells	130-090-489
	APC	100 tests with 10 <sup>6</sup> cells	130-090-939
Mouse IL-17 Secretion Assay – Detection Kit	PE	100 tests with 10 <sup>6</sup> cells	<i>coming soon</i>
	APC	100 tests with 10 <sup>6</sup> cells	<i>coming soon</i>

## Antibodies for intracellular cytokine staining

Product	Clone, isotype	Conjugate	Capacity	Order no.
Anti-IFN-α	LT27:295, mouse IgG1	FITC	100 tests with 10 <sup>7</sup> cells	130-092-600
		PE	100 tests with 10 <sup>7</sup> cells	130-092-601
		APC	100 tests with 10 <sup>7</sup> cells	130-092-602
		pure	100 µg in 1 mL	130-092-604
Anti-IFN-γ	45-15, mouse IgG1	FITC	100 tests with 10 <sup>7</sup> cells	130-091-641
		PE	100 tests with 10 <sup>7</sup> cells	130-091-653
		APC	100 tests with 10 <sup>7</sup> cells	130-091-640
Anti-IL-2	N7.48A, mouse IgG2a	PE	100 tests with 10 <sup>7</sup> cells	130-091-646
		APC	100 tests with 10 <sup>7</sup> cells	130-091-644
Anti-IL-4	7A3-3, mouse IgG1	PE	100 tests with 10 <sup>7</sup> cells	130-091-647
Anti-IL-5	JES1-39D10, rat IgG2a	PE	100 tests with 10 <sup>7</sup> cells	130-091-648
		APC	100 tests with 10 <sup>7</sup> cells	130-091-834
Anti-IL-10	B-T10, mouse IgG1	PE	100 tests with 10 <sup>7</sup> cells	130-091-643
		APC	100 tests with 10 <sup>7</sup> cells	130-091-642
Anti-IL-12 (p40/p70)	8.6, mouse IgG1	PE	100 tests with 10 <sup>7</sup> cells	130-092-774
		APC	100 tests with 10 <sup>7</sup> cells	130-092-775
Anti-IL-13	JES10-5A2.2, rat IgG1	PE	100 tests with 10 <sup>7</sup> cells	130-092-964
Anti-TNF-α	cA2, human IgG1	FITC	100 tests with 10 <sup>7</sup> cells	130-091-650
		PE	100 tests with 10 <sup>7</sup> cells	130-091-651
		APC	100 tests with 10 <sup>7</sup> cells	130-091-649
<b>Non-human primate cells</b>				
Anti-IFN-γ	45-15, mouse IgG1	FITC	100 tests with 10 <sup>7</sup> cells	130-091-641
		PE	100 tests with 10 <sup>7</sup> cells	130-091-653
		APC	100 tests with 10 <sup>7</sup> cells	130-091-640
Anti-IL-2	N7.48A, mouse IgG2a	PE	100 tests with 10 <sup>7</sup> cells	130-091-646
		APC	100 tests with 10 <sup>7</sup> cells	130-091-644

# Product overview

## Non-human primate cells

Anti-IL-4	7A3-3, mouse IgG1	PE	100 tests with 10 <sup>7</sup> cells	130-091-647
Anti-IL-5	JES1-39D10, rat IgG2a	PE	100 tests with 10 <sup>7</sup> cells	130-091-648
		APC	100 tests with 10 <sup>7</sup> cells	130-091-834
Anti-IL-10	B-T10, mouse IgG1	PE	100 tests with 10 <sup>7</sup> cells	130-091-643
		APC	100 tests with 10 <sup>7</sup> cells	130-091-642
Anti-IL-12 (p40/p70)	8.6, mouse IgG1	PE	100 tests with 10 <sup>7</sup> cells	130-092-774
		APC	100 tests with 10 <sup>7</sup> cells	130-092-775
Anti-IL-13	JES10-5A2.2, rat IgG1	PE	100 tests with 10 <sup>7</sup> cells	130-092-964

## Mouse cells

Anti-IFN-γ	AN18.17.24, rat IgG	PE	100 tests with 10 <sup>7</sup> cells	130-092-346
		APC	100 tests with 10 <sup>7</sup> cells	130-092-347
Anti-IL-2	JES6-5H4, rat IgG2b	PE	100 tests with 10 <sup>7</sup> cells	130-092-302
		APC	100 tests with 10 <sup>7</sup> cells	130-092-303
Anti-IL-17	TC11-18H10, rat IgG1	FITC	100 tests with 10 <sup>7</sup> cells	<i>coming soon</i>
		PE	100 tests with 10 <sup>7</sup> cells	<i>coming soon</i>
		APC	100 tests with 10 <sup>7</sup> cells	<i>coming soon</i>
Anti-TNF-α	MP6-XT22, rat IgG1	FITC	100 tests with 10 <sup>7</sup> cells	130-092-244
		PE	100 tests with 10 <sup>7</sup> cells	130-092-245
		APC	100 tests with 10 <sup>7</sup> cells	130-092-246

## Intracellular Cytokine Detection Kits

Product	Capacity	Order no.
<b>Human cells</b>		
CD154/IFN-γ/CD4 Detection Kit	100 tests with 10 <sup>6</sup> cells	130-092-814
CD154/IL-2/CD4 Detection Kit	100 tests with 10 <sup>6</sup> cells	130-092-818
CD154/IL-4/CD4 Detection Kit	100 tests with 10 <sup>6</sup> cells	130-092-817
CD154/IL-5/CD4 Detection Kit	100 tests with 10 <sup>6</sup> cells	130-092-812
CD154/TNF-α/CD4 Detection Kit	100 tests with 10 <sup>6</sup> cells	130-092-813

## Products for detection and enrichment of CD137<sup>+</sup> and CD154<sup>+</sup> cells

Product	Clone, isotype	Capacity	Order no.
<b>Human cells</b>			
CD137 MicroBead Kit, human		For 10 <sup>9</sup> total cells	130-093-476
CD154 MicroBead Kit, human		For 10 <sup>9</sup> total cells	130-092-658
CD137-PE, human	4B4-1, mouse IgG1	100 tests with 10 <sup>7</sup> cells	130-093-475
CD154-PE, human	5C8, mouse IgG2A	100 tests with 10 <sup>7</sup> cells	130-092-289
CD154-APC, human	5C8, mouse IgG2A	100 tests with 10 <sup>7</sup> cells	130-092-290
CD154-Biotin, human	5C8, mouse IgG2A	100 tests with 10 <sup>7</sup> cells	130-092-690

## Mouse cells

CD154 Enrichment and Detection Kit (PE), mouse		For 10 <sup>9</sup> total cells	130-093-129
CD154-PE, mouse	MR1, hamster IgG3	100 tests with 10 <sup>7</sup> cells	130-092-106
CD154-APC, mouse	MR1, hamster IgG3	100 tests with 10 <sup>7</sup> cells	130-092-105
CD154-Biotin, mouse	MR1, hamster IgG3	100 tests with 10 <sup>7</sup> cells	130-092-104
CD154 Detection Cocktail (PE), mouse		100 tests with 10 <sup>6</sup> cells	130-093-084
CD154 Detection Cocktail (APC), mouse		100 tests with 10 <sup>6</sup> cells	130-093-083

## Cell stimulation reagents

Product	Quantity/capacity	Short description	Order no.
T Cell Activation/Expansion Kit, human		Activation and/or expansion of human T cells	130-091-441
T Cell Activation/Expansion Kit, non-human primate		Activation and/or expansion of rhesus monkey T cells	130-092-919
CytoStim, human	200 µL for 10 <sup>8</sup> total cells	Rapid and efficient restimulation of human effector/memory T cells	130-092-172
CytoStim, human	1 mL for 5 × 10 <sup>8</sup> total cells	Rapid and efficient restimulation of human effector/memory T cells	130-092-173
CMV pp65 – Recombinant Protein, human	200 µL for 10 <sup>8</sup> total cells	<i>In vitro</i> stimulation of CMV pp65-specific T cells	130-091-824
CMV pp65 – Recombinant Protein, human	2 × 1 mL for 10 <sup>9</sup> total cells	<i>In vitro</i> stimulation of CMV pp65-specific T cells	130-091-823
PepTivator – CMV pp65, human	6 nmol/peptide for 10 <sup>8</sup> total cells	CMV pp65 peptide pool for efficient <i>in vitro</i> stimulation of human CMV pp65-specific CD4 <sup>+</sup> and CD8 <sup>+</sup> T cells	130-093-438
PepTivator – CMV pp65, human	60 nmol/peptide for 10 <sup>9</sup> total cells	CMV pp65 peptide pool for efficient <i>in vitro</i> stimulation of human CMV pp65-specific CD4 <sup>+</sup> and CD8 <sup>+</sup> T cells	130-093-435
PepTivator – CMV IE-1, human	6 nmol/peptide for 10 <sup>8</sup> total cells	CMV IE-1 peptide pool for efficient <i>in vitro</i> stimulation of human CMV IE-1-specific CD4 <sup>+</sup> and CD8 <sup>+</sup> T cells	130-093-493
PepTivator – CMV IE-1, human	60 nmol/peptide for 10 <sup>9</sup> total cells	CMV IE-1 peptide pool for efficient <i>in vitro</i> stimulation of human CMV IE-1-specific CD4 <sup>+</sup> and CD8 <sup>+</sup> T cells	130-093-494
PepTivator – Adv5 Hexon, human	6 nmol/peptide for 10 <sup>8</sup> total cells	Adv5 hexon peptide pool for efficient <i>in vitro</i> stimulation of human Adv5 hexon-specific CD4 <sup>+</sup> and CD8 <sup>+</sup> T cells	130-093-495
PepTivator – Adv5 Hexon, human	60 nmol/peptide for 10 <sup>9</sup> total cells	Adv5 hexon peptide pool for efficient <i>in vitro</i> stimulation of human Adv5 hexon-specific CD4 <sup>+</sup> and CD8 <sup>+</sup> T cells	130-093-496
PepTivator – EBV EBNA-1, human	6 nmol/peptide for 10 <sup>8</sup> total cells	EBV EBNA-1 peptide pool for efficient <i>in vitro</i> stimulation of human EBV EBNA-1-specific CD4 <sup>+</sup> and CD8 <sup>+</sup> T cells	coming soon
PepTivator – EBV EBNA-1, human	60 nmol/peptide for 10 <sup>9</sup> total cells	EBV EBNA-1 peptide pool for efficient <i>in vitro</i> stimulation of human EBV EBNA-1-specific CD4 <sup>+</sup> and CD8 <sup>+</sup> T cells	coming soon

## Related products

Product	Description	Order no.
MACSmix™ Tube Rotator	Tube rotator working independently of a permanent power supply	130-090-753



Miltenyi Biotec

130-093-492.02

**Miltenyi Biotec GmbH**  
Friedrich-Ebert-Straße 68  
51429 Bergisch Gladbach  
Germany  
Phone +49 2204 8306-0  
Fax +49 2204 85197  
macs@miltenyibiotec.de

**Miltenyi Biotec Inc.**  
12740 Earhart Avenue  
Auburn, CA 95602, USA  
Phone 800 FOR MACS,  
+1 530 888 8871  
Fax +1 530 888 8925  
macs@miltenyibiotec.com

**Miltenyi Biotec  
Australia Pty. Ltd.**  
Phone +61 02 8877 7400  
macs@miltenyibiotec.com.au

**Miltenyi Biotec B.V. (Benelux)**  
macs@miltenyibiotec.nl  
**Customer service Netherlands**  
Phone 0800 4020120  
**Customer service Belgium**  
Phone 0800 94016  
**Customer service Luxembourg**  
Phone 800 24971

**Miltenyi Biotec Trading  
(Shanghai) Co., Ltd. (P.R. China)**  
Phone +86 21 6235 1005  
macs@miltenyibiotec.com.cn

**Miltenyi Biotec SAS (France)**  
Phone +33 1 56 98 16 16  
macs@miltenyibiotec.fr

**Miltenyi Biotec S.r.l. (Italy)**  
Phone +39 051 646 0411  
macs@miltenyibiotec.it

**Miltenyi Biotec K.K. (Japan)**  
Phone +81 3 5646 8910  
macs@miltenyibiotec.jp

**Miltenyi Biotec Asia Pacific  
Pte. Ltd. (Singapore)**  
Phone +65 6238 8183  
macs@miltenyibiotec.com.sg

**Miltenyi Biotec S.L. (Spain)**  
Phone +34 91 512 12 90  
macs@miltenyibiotec.es

**Miltenyi Biotec Ltd. (UK)**  
Phone +44 1483 799 800  
macs@miltenyibiotec.co.uk

[www.miltenyibiotec.com](http://www.miltenyibiotec.com)