MACS
Miltenyi Biotec

Regulatory T cell research

Unique cell separation kits

Flow cytometric analysis

Efficient cell expansion

Convenient functional assays

MACS® Technology by Miltenyi Biotec

Comprehensive solutions from cell to molecular analysis

Since its introduction in 1989, MACS® Technology has become the gold standard for cell separation. Nowadays, Miltenyi Biotec stands for more than cell separation, offering over 1000 innovative research products for biomedical research and life sciences. The MACS Product portfolio includes instruments and reagents for sample preparation, cell separation, cell analysis, cell culture, and molecular biology. Over the last 20 years, researchers have published more than 14,500 papers with our products. Miltenyi Biotec has a strong commitment to constantly develop new products for current and future research.

MACS Sample Preparation

The quality of an experiment strictly depends on the quality of the sample preparation. Miltenyi Biotec offers an innovative instrument, the gentleMACS™ Dissociator, for fast and gentle sample preparation from solid tissues as well as cultured cells. Specific programs and tubes were developed for molecular applications.

MACS Cell Separation

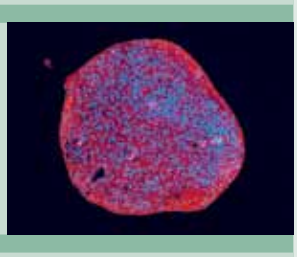
A large panel of MACS MicroBeads and MicroBead Kits are available for the isolation of virtually any cell type. The cells can be separated manually or automatically. The autoMACS® Pro Separator was designed for automated cell sorting of multiple samples.

MACS Cell Analysis

Miltenyi Biotec provides a large selection of monoclonal antibodies and kits for fluorescence microscopy and flow cytometry. The innovative MACSQuant® Analyzer is an extremely compact, easy-to-use benchtop flow cytometer. The instrument is fully automated and enables absolute cell counting.

MACS Cell Culture

The product portfolio for cell culture includes media as well as recombinant cytokines and growth factors up to GMP grade.



MACSmolecular

Miltenyi Biotec provides products for protein isolation and detection, mRNA purification and amplification, cDNA synthesis and labeling, microRNA analysis as well as microarray technologies and instrumentation. Also on offer are genomic services: gene and microRNA expression analyses, array-CGH, and bioinformatics.

MACS Cell Separation

Providing a firm foundation for reliable results

Tried and tested

MACS® Technology is the recognized standard in cell separation. Numerous publications have proven its versatility for many applications in both basic research and clinical arenas. Rare cells, abundant cells, or sophisticated cell subsets can be easily and reliably sorted using MACS Technology. Miltenyi Biotec provides researchers worldwide with the tools for high-quality separations.

MACS MicroBeads

MACS® Technology is based on MACS MicroBeads, MACS Separators, and MACS Columns. MACS MicroBeads are superparamagnetic particles of approximately 50 nanometers in diameter. They are composed of a biodegradable matrix, and it is therefore not necessary to remove them from cells after the separation process.

- Specific monoclonal antibody conjugates
- Colloidal, for easy handling and short incubation times (15 minutes)
- Small (50 nm), non-toxic, biodegradable
- Detachment is not required for downstream experiments

MACS MicroBeads do not alter the structure, function, or activity status of labeled cells and are not known to interfere with subsequent experiments.

How you benefit from MACS Technology

- Optimal recovery and excellent purity
- Easy separation of large cell numbers or rare cells
- Fast, convenient, and absolutely reliable
- Gentle to cells
- Automated cell separation with the autoMACS® Pro Separator
- Compatible with flow cytometry

Contents

The convenient route to separation

Isolate human or mouse Treg cells

Separate Treg cells with high purity

Miltenyi Biotec offers a variety of kits for the isolation of human, mouse, and non-human primate Treg cells based on MACS® Technology and specific antibodies. The isolated Treg cells can then be used for functional assays and also for adoptive transfer in mouse models.

CD4$^+$CD25$^+$ Regulatory T Cell Isolation Kits for human or mouse

These kits provide a convenient way to isolate functional CD4$^+$CD25$^+$ Treg cells with high expression levels of FoxP3. Additionally, the cell separation results in CD4$^+$CD25$^-$ T cells that can be used as effector cells in suppression assays [1,2].

Treg cells isolated with the CD4$^+$CD25$^+$ Regulatory T Cell Isolation Kit, human, were used to investigate their role in:

- autoimmune diseases [3]
- antitumor immunity [1,4,5]
- dendritic cell vaccination [2]
- allergy [6]
- infectious diseases [7]

Treg cells isolated with the CD4$^+$CD25$^+$ Regulatory T Cell Isolation Kit, mouse, have been used in various mouse models for the following topics:

- adoptive transfer [8]
- autoimmunity [9,10]
- melanoma [11]
- *in vitro* suppression assays [12]

For order information see page 12.

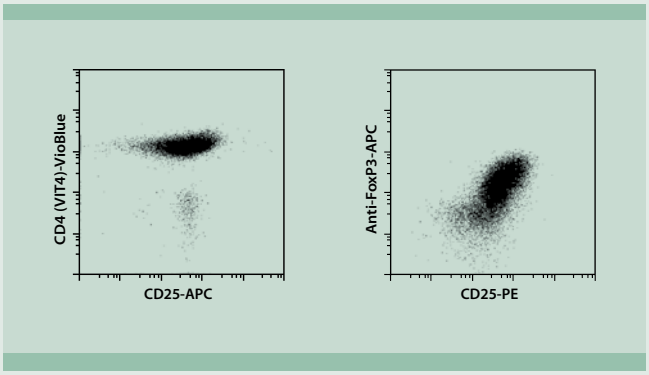


Figure 1: CD4$^+$CD25$^+$ Treg cells isolated from human PBMCs using the CD4$^+$CD25$^+$ Regulatory T Cell Isolation Kit
The cells were stained with CD25-APC and CD4 (VIT4)-VioBlue or CD25-PE and Anti-FoxP3-APC. Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. Data showing FoxP3 staining were gated according to CD4 expression.

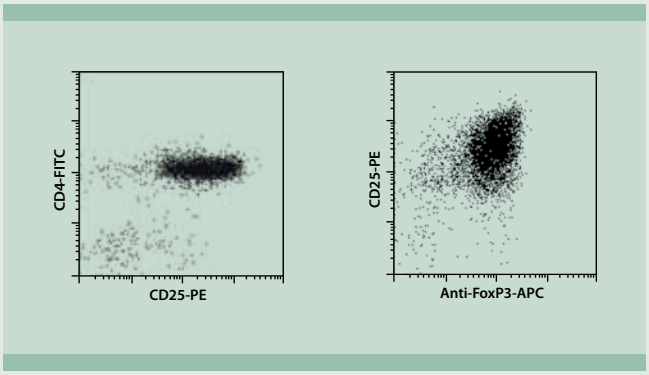


Figure 2: Treg cells isolated from mouse spleen using the CD4$^+$CD25$^+$ Regulatory T Cell Isolation Kit, mouse
Cells were stained with CD25-PE and CD4-FITC or Anti-FoxP3-APC and analyzed by flow cytometry.

Higher purity and excellent recovery

Additional discrimination of Treg cells

Further markers for greater purity

There are several additional markers that can be used to discriminate between Treg and other T cells:

$CD4^+CD25^+CD127^{dim/-}$ Regulatory T Cell Isolation Kit II, human

CD127 is expressed on the majority of mature T cells. However it is absent on Treg cells and its expression is inversely correlated with FoxP3 expression [13,14].

- The new $CD4^+CD25^+CD127^{dim/-}$ Regulatory T Cell Isolation Kit II thus allows enhanced purity of Treg cells [15,16].

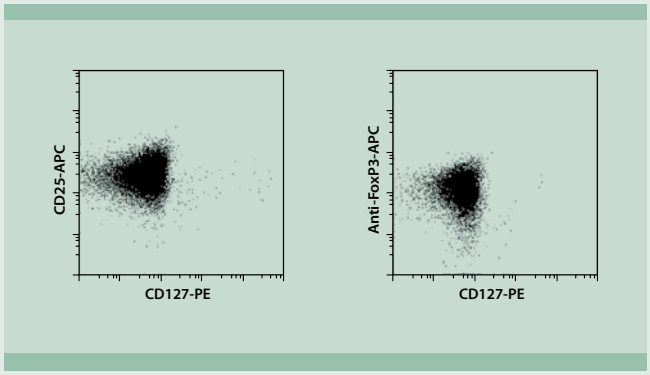


Figure 3: $CD4^+CD25^+CD127^{dim/-}$ Treg cells isolated from human PBMCs using the $CD4^+CD25^+CD127^{dim/-}$ Regulatory T Cell Isolation Kit II
The cells were stained with CD4-FITC, CD25-APC, and CD127-PE (left), or CD4-FITC, CD127-PE, and Anti-FoxP3-APC (right), and analyzed by flow cytometry using the MACSQuant® Analyzer. Gating was performed according to CD4 expression.

$CD4^+CD25^+CD45RA^+$ Regulatory T Cell Isolation Kit, human

Treg cells isolated from the $CD45RA^+$ naïve T cell compartment have been shown to be optimal for *in vitro* expansion.

- Treg cells isolated with the $CD4^+CD25^+CD45RA^+$ Regulatory T Cell Isolation Kit and then expanded *in vitro* maintained the $FoxP3^+$ phenotype and their suppressive function [17,18].

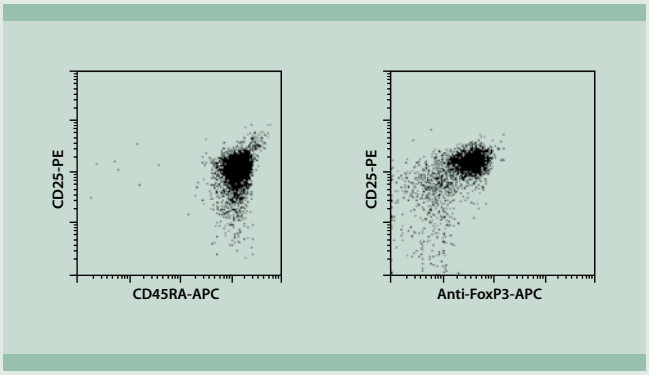


Figure 4: $CD4^+CD25^+CD45RA^+$ Treg cells isolated from human PBMCs using the $CD4^+CD25^+CD45RA^+$ Regulatory T Cell Isolation Kit
The cells were fluorescently stained with CD4-FITC, CD25-PE, and either CD45RA-APC (left) or Anti-FoxP3-APC (right), and gated on viable $CD4^+$ lymphocytes.

$CD25^+CD49d^-$ Regulatory T cell Isolation Kit, human

The $CD49d^+$ antigen is present on the surface of most pro-inflammatory effector T cells.

- Within the $CD25^+$ T cell subset the CD49d marker can be used to deplete contaminating effector T cells which only transiently express FoxP3 and CD25 [19].
- In addition, most naïve Treg cells are positive for CD49d, so these can be depleted to leave a population of highly suppressive Treg cells.

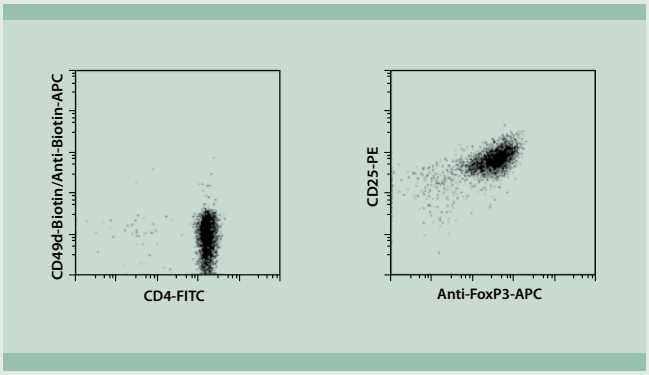


Figure 5: $CD25^+CD49d^-$ Treg cells isolated from human PBMCs using the $CD25^+CD49d^-$ Regulatory T Cell Isolation Kit
Cells were stained with CD4-FITC, CD49d-Biotin, and Anti-Biotin-APC (left), or CD4-FITC, CD25-PE, and Anti-FoxP3-APC (right) and analyzed by flow cytometry using the MACSQuant® Analyzer. Gating was performed according to CD4 expression (right).

Secretion is the key

Study Tr1 and Breg cells, human and mouse

Secretion of cytokines

Cytokine secretion assay technology (see figure 7)—a unique tool for analysis and isolation of viable IL-2- and IL-10–secreting type 1 regulatory T cells (Tr1 cells).

Different types of Treg cells can be induced from naïve T cells in the periphery: Tr1 cells are characterized by their ability to produce IL-10 and TGF-ß and are FoxP3-negative.

The IL-10 Secretion Assay, human has been used for:

- enumeration of Tr1 cells in patients with autoimmune skin disease [20]
- characterization of allergen-specific Tr1 cells [21]
- studies on the effect of allergen exposure on Tr1 cells [22]
- studies on memory T cell subsets [23]

The IL-10 Secretion Assay, mouse has been used for:

- the study of transplantation tolerance with isolated Tr1 cells [24]

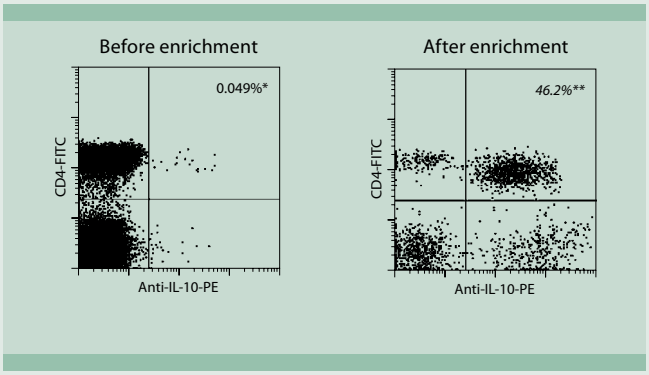


Figure 6: Enrichment of IL-10–secreting cells from CMV lysate–stimulated PBMCs of a CMV⁺ donor
Cells were stimulated for 16 hours. The responding cells were stained and isolated according to secretion of IL-10 using the IL-10 Secretion Assay – Cell Enrichment and Detection Kit.
* Percentage represents frequency among CD4⁺ T cells.
** Percentage represents frequency among enriched cells.

For order information see page 12.

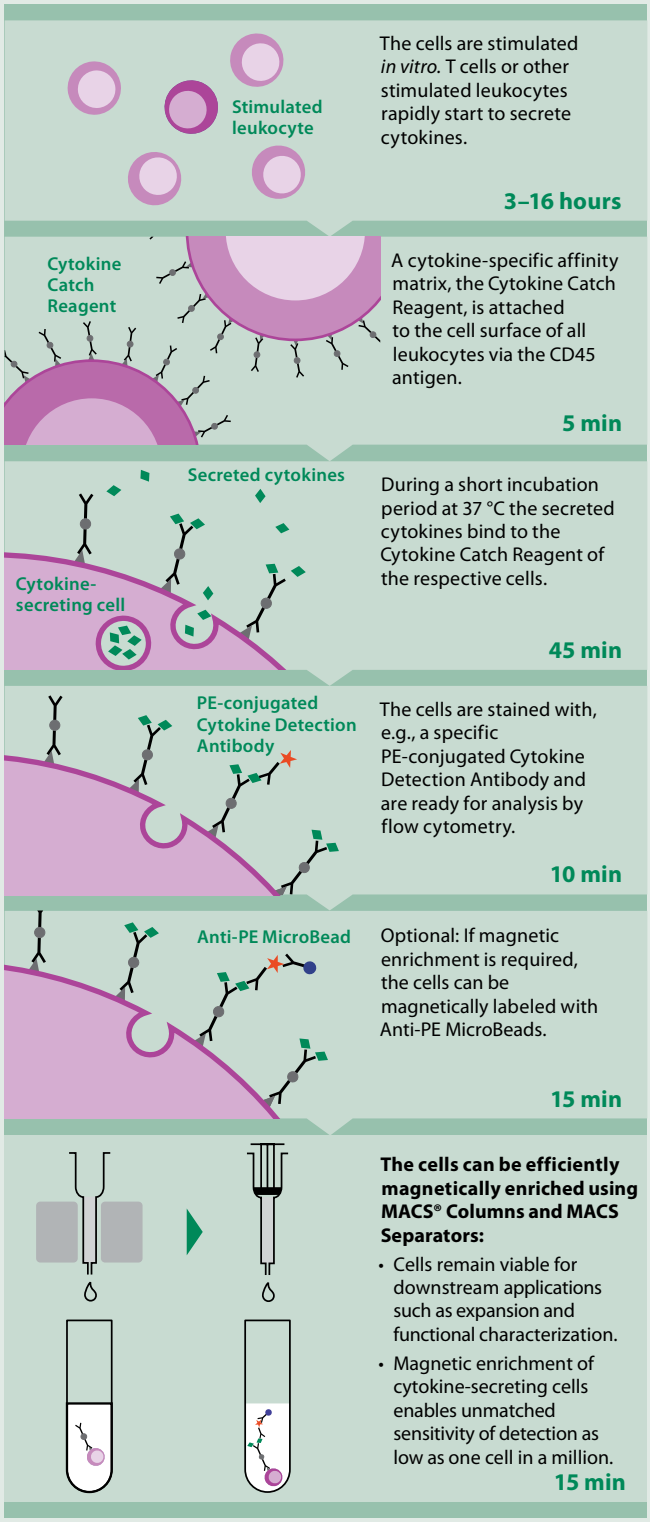


Figure 7: Cytokine secretion assays—the principle

From Treg isolation to functional analysis

Innovative Tools for Treg research

Overview of tools for the isolation and analysis of human Treg cells

Natural Treg (nTreg) cells					Induced Treg (iTreg) cells, Tr1
Phenotype of target cells	$CD4^+CD25^+$	$CD4^+CD25^+CD127^{dim/-}$	$CD4^+CD25^+CD49d^-$	$CD4^+CD25^+CD45RA^+$	$IL\text{-}10^+$
MACS® Product	$CD4^+CD25^+$ Regulatory T Cell Isolation Kit	$CD4^+CD25^+CD127^{dim/-}$ Regulatory T Cell Isolation Kit II	$CD25^+CD49d^-$ Regulatory T Cell Isolation Kit	$CD4^+CD25^+CD45RA^+$ Regulatory T Cell Isolation Kit	IL-10 Secretion Assay – Cell Enrichment and Detection Kit (PE)
Kit components for depletion	Cocktail of biotinylated antibodies: CD8, CD14, CD16, CD19, CD36, CD56, CD123, Anti-TCRγ/δ, CD235a; Anti-Biotin MicroBeads	Cocktail of biotinylated antibodies: CD8, CD19, CD123, CD127; Anti-Biotin MicroBeads	CD8 and CD49d MicroBeads	Cocktail of biotinylated antibodies: CD8, CD14, CD16, CD19, CD36, CD45RO, CD56, CD123, Anti-TCRγ/δ, CD235a; Anti-Biotin MicroBeads	
Kit components for positive selection	CD25 MicroBeads II	CD25 MicroBeads II	CD25 MicroBeads II	CD25 MicroBeads II	IL-10 Secretion Assay – Cell Enrichment and Detection Kit (PE)
Other associated phenotypes of interest	$FoxP3^+$, $GITR^+$, $CD39^{+/-}$, $CD49d^{+/-}$, $CD127^{+/-}$,	$FoxP3^+$, $GITR^+$, $CD39^{+/-}$, $CD49d^{+/-}$, $CD127^-$	$FoxP3^+$, $GITR^+$, $CD39^{+/-}$, $CD45RO^+$, $CD49d^-$, $CD127^{+/-}$	$FoxP3^+$, $GITR^+$, $CD39^{+/-}$, $CD45RO^-$, $CD49d^-$, $CD127^-$	$FoxP3^-$
Tool for functional assays	Treg Suppression Inspector	Treg Suppression Inspector	Treg Suppression Inspector	Treg Suppression Inspector	
Suppression mechanism	Various mechanisms described for *in vitro* and *in vivo* suppression	Various mechanisms described for *in vitro* and *in vivo* suppression	Various mechanisms described for *in vitro* and *in vivo* suppression	Various mechanisms described for *in vitro* and *in vivo* suppression	IL-10
Applications	• Isolation of $CD4^+CD25^+$ and $CD4^+CD25^-$ cells • Ideal for functional analysis using the Treg Suppression Inspector	• Excellent purity and recovery of $FoxP3^+$ cells • For *in vitro* expansion	• Isolation of $CD4^+CD25^+$ Treg cells depleted of $CD25^+CD49d^+$ effector T cells and most naive Treg cells • For highly suppressive Treg cells	• Isolation of naive Treg cells • Stable expression of FoxP3 • Ideal for long-term expansion of Treg cells	Enumeration, expansion, and functional analysis of IL-10–producing T cells

Shed more light on Treg cells

Convenient analysis by flow cytometry

Surface and intracellular staining

Treg Detection Kits for human, non-human primate, and mouse

These kits contain antibodies and reagents for staining and detection of Treg cells by flow cytometry. The kits include a special FoxP3 Staining Buffer Set, FcR Blocking Reagent, and an optimized protocol.

- Cell surface staining of CD4 and CD25
- Intracellular staining of FoxP3

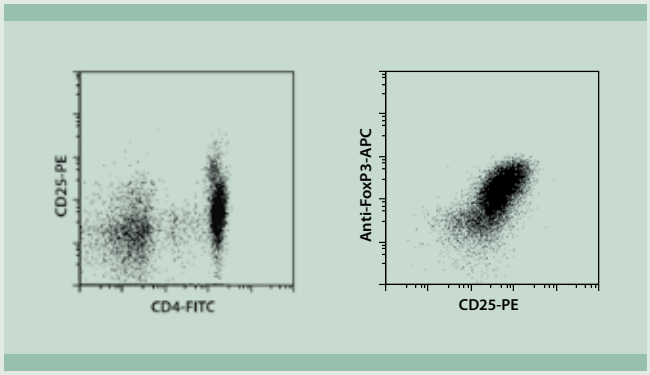


Figure 8: Human PBMCs stained with the Treg Detection Kit (APC)
Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. For the dot plot that shows CD25-PE and Anti-FoxP3-APC staining, gating was performed according to CD4 expression.

Detection of Treg cell surface markers

Miltenyi Biotec's product portfolio is continuously expanding to include tools for new relevant markers, e.g., the new CD127 antibodies that define the Treg population with even greater precision. On Treg cells CD127 is absent and its expression inversely correlates with FoxP3 expression. [25,26]

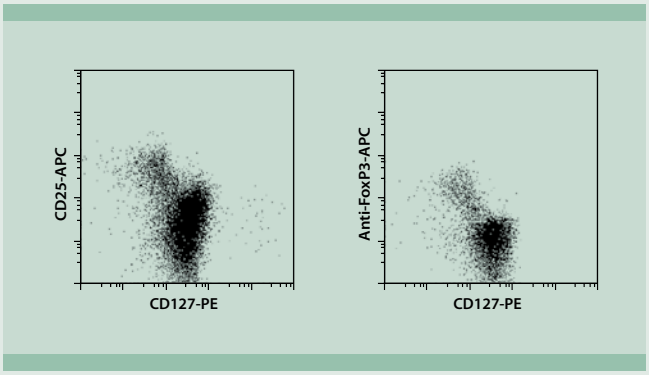


Figure 9: Human PBMCs stained with CD127-PE and CD25-APC or CD127-PE and Anti-FoxP3-APC
Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. Gating was performed according to CD4 expression.

For order information see pages 12 and 13.

Purely functional

Study Treg function in a convenient assay

Human Treg cell–mediated suppression of responder T cells *in vitro*

Treg Suppression Inspector, human

The Treg Suppression Inspector offers the ideal solution for the functional analysis of human Treg cells.
It is based on Anti-Biotin MACSiBead™ Particles as a T cell–stimulating reagent and suppression by Treg cells [27]. The Treg Suppression Inspector stimulates the proliferation of responder T cells ($CD4^+CD25^-$ or $CD4^+$ T cells). Coculture of Treg cells with responder T cells in the presence of the Treg Suppression Inspector results in reduced proliferation of the responder T cells due to the suppressive function of the Treg cells.

Used to characterize Treg cell suppression activity:

- in antitumor immunity [28]
- after dendritic cell vaccination [29]

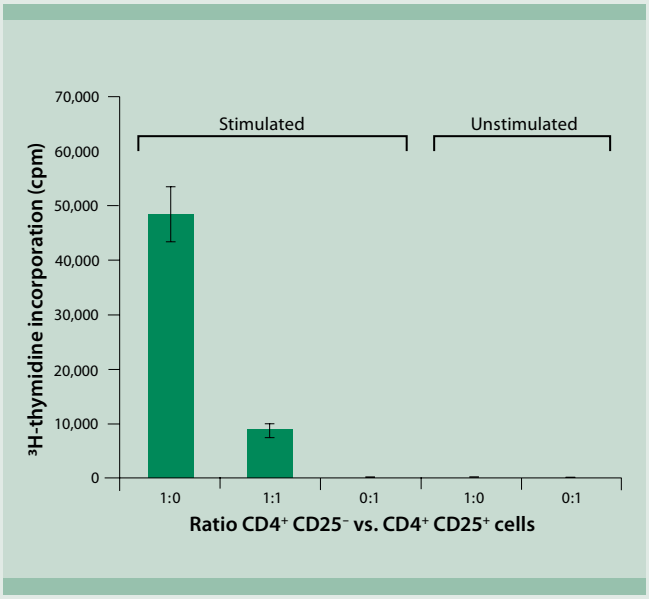


Figure 10: Treg suppression assay using the Treg Suppression Inspector
Human Treg cells, isolated with the $CD4^+CD25^+$ Regulatory T Cell Isolation Kit, were cocultured with $CD4^+CD25^-$ responder T cells at different ratios. For T cell stimulation, the Treg Suppression Inspector was added to the culture. As controls, Treg and responder T cells alone were cultured without any stimulus. Proliferation of T cells was determined by measuring ^{3}H-thymidine incorporation.

For order information see page 14.

Treg Expansion Kit, human + mouse

The Treg Expansion Kit, human is based on MACSiBead Particles for the expansion of human Treg cells after isolation with the $CD4^+CD25^+CD127^{dim/-}$ or $CD4^+CD25^+CD45RA^+$ Regulatory T Cell Isolation Kits. The Kit is designed to efficiently expand Treg cells and to maintain FoxP3 expression.

Progress through expression analysis

Molecular characterization of Treg cells

Miltenyi Biotec offers a variety of genomic services and provides a number of unique tools for the characterization of Treg cells.

Isolation of mRNA and cDNA synthesis

The µMACS™ One-step cDNA Kit was used to analyze FoxP3 expression in $CD4^+CD25^+$ Treg cells and in $CD4^+CD25^-$ T cells from patients with malignant glioma [30,31].

The µMACS One-step cDNA Kit

- One-step mRNA isolation and cDNA synthesis
- Outstanding sensitivity and high purity
- Five cells are sufficient for PCR analysis

microRNA expression analysis

Microarray analysis has shown that FoxP3 directly controls microRNA expression in Treg cell formation and function [32,33].

miRXplore™ Microarrays

- Gold standard expression profiling
- Arrays include current mature human, mouse, rat, and viral microRNAs
- Sequences fully match miRBase database
- Designed and developed in cooperation with leading microRNA scientists at Rockefeller University [34]

Cell type-specific total RNA

Total RNA from human $CD4^+CD25^+$ Treg cells

- High-quality RNA for gene expression analysis
- Use for quantitative RT-PCR or microarray analysis

Genomic services for RNA research—expertise at your service

Send sample—receive results

Technical support for experimental design and microarray selection
- microRNA miRXplore™ Microarrays
- Topic-defined, custom PIQOR™ Microarrays
- Agilent Whole Genome Microarrays

RNA extraction and quality control

Optional:
- **Amplification and quality control**
- **SuperAmp Service*: 1–100,000 cells**

Synthesis and purification of fluorescently labeled probes

Microarray hybridization

Image capture and analysis of primary data

Optional: **Bioinformatics Services****
Pre-processing, Ratio Building, Cluster, Discriminatory Genes, Functional Grouping, Pathway Analysis

Results and report
Data on CD-ROM

* microRNAs cannot be amplified with the SuperAmp Service.
** Please inquire for microRNA Bioinformatics Services.

Complete customer care

Trusted worldwide support from Miltenyi Biotec

Support when and where it's needed

Miltenyi Biotec is committed to providing outstanding customer care worldwide.

Sales support

All Miltenyi Biotec technical sales consultants have a background in life sciences and can offer advice on:

- experimental design
- troubleshooting experimental protocols
- choosing the appropriate Miltenyi Biotec product

Technical Support

Worldwide technical support can be accessed by:

- e-mail or telephone: simply ask technical support a question
- an online discussion forum
- a list of frequently asked questions
- a comprehensive library of datasheets and protocols

Learning center

Training resources are adapted to the individual:

- hands-on training offered onsite or at dedicated Miltenyi Biotec facilities
- online multimedia presentations
- webinars provide real-time access to a Miltenyi Biotec expert

Instrument service

A comprehensive range of service and support agreements have been developed to ensure that laboratory instruments maintain a high performance.

Visit **www.miltenyibiotec.com/support** for more details.

Order information

Place your order by fax, phone, or online!

Products for the isolation and analysis of Treg cells

Product	Capacity	Order no.
Human cells		
$CD4^+CD25^+$ Regulatory T Cell Isolation Kit, human	For 10^9 total cells	130-091-301
$CD4^+CD25^+CD127^{dim/-}$ Regulatory T Cell Isolation Kit II, human	For 10^9 total cells	130-094-775
$CD4^+CD25^+CD45RA^+$ Regulatory T Cell Isolation Kit, human	For 2×10^9 total cells	130-093-631
$CD25^+CD49d^-$ Regulatory T Cell Isolation Kit, human	For 10^9 total cells	130-094-551
CD127 MicroBead Kit, human	For 10^9 total cells	130-094-945
Treg Detection Kit (CD4/CD25/CD127)	25 tests	130-096-076
Treg Detection Kit (CD4/CD25/CD127)	100 tests	130-096-082
Treg Detection Kit (CD4/CD25/FoxP3) (PE)	100 tests	130-094-163
Treg Detection Kit (CD4/CD25/FoxP3) (APC)	100 tests	130-094-158
Non-human primate cells		
$CD4^+CD25^+$ Regulatory T Cell Isolation Kit, non-human primate	For 10^9 total cells	130-092-984
Mouse cells		
$CD4^+CD25^+$ Regulatory T Cell Isolation Kit, mouse	For 10^9 total cells	130-091-041
Treg Detection Kit (CD4/CD25/FoxP3) (PE)	100 tests	130-094-165
Treg Detection Kit (CD4/CD25/FoxP3) (APC)	100 tests	130-094-164

Cytokine secretion assays

Product	Order no. Cell Enrichment and Detection Kit	Order no. Detection Kit
Human cells		
Large scale IFN-γ Secretion Assay – Enrichment Kit	(PE) 130-091-329	
IFN-γ Secretion Assay	(PE) 130-054-201	(FITC) 130-090-433, (PE) 130-054-202 (APC) 130-090-762
IL-2 Secretion Assay	(PE) 130-090-488	(PE) 130-090-487, (APC) 130-090-763
IL-10 Secretion Assay	(PE) 130-090-435	(PE) 130-090-434, (APC) 130-090-761
IL-17 Secretion Assay	(PE) 130-094-542	(PE) 130-094-537, (APC) 130-094-536
Mouse cells		
Mouse IFN-γ Secretion Assay	(PE) 130-090-517	(PE) 130-090-516, (APC) 130-090-984
Mouse IL-2 Secretion Assay	(PE) 130-090-492	(PE) 130-090-491, (APC) 130-090-987
Mouse IL-10 Secretion Assay	(PE) 130-090-490	(PE) 130-090-489
Mouse IL-17 Secretion Assay	(PE) 130-094-213	(PE) 130-094-205, (APC) 130-094-207

Products for flow cytometric analysis of Treg cells

Product	Clone, isotype	Order no.
Human cells		
Anti-FoxP3-PE, -APC	3G3, mouse IgG1	130-093-014, 130-093-013
FoxP3 Staining Buffer Set		130-093-142
Anti-GITR-PE, -Biotin, pure, pure – functional grade	DT5D3, mouse IgG1	130-092-895, 130-092-886, 130-092-885, 130-093-052
CD4 (VIT4)-FITC, -PE, -APC, -VioBlue, -PerCP	VIT44, mouse IgG2a	130-092-358, 130-092-373, 130-092-374, 130-094-153, 130-094-963
CD25-PE, -APC, -Biotin	4E3, mouse IgG2b	130-091-024, 130-092-858, 130-091-235
CD39-FITC, -PE, -APC, -Biotin, pure	MZ18-23C8, mouse IgG1	130-093-502, 130-093-503, 130-093-504, 130-093-505, 130-093-506
CD49d-FITC, -PE, -APC, -Biotin, pure	MZ18-24A9, mouse IgG2b	130-093-283, 130-093-282, 130-093-281, 130-093-280, 130-093-279
CD127-FITC, -PE, -APC, -Biotin, pure	MB15-18C9, mouse IgG2a	130-094-888, 130-094-889, 130-094-890, 130-094-891, 130-094-942
Non-human primate cells		
Anti-FoxP3-PE, -APC	3G3, mouse IgG1	130-093-014, 130-093-013
FoxP3 Staining Buffer Set		130-093-142
CD4-FITC, -PE, -APC	M-T466, mouse IgG1	130-090-501, 130-091-231, 130-091-232
CD25-PE, -APC, -Biotin	4E3, mouse IgG2b	130-091-024, 130-092-858, 130-091-235
CD39-FITC, -PE, -APC, -Biotin, pure	MZ18-23C8, mouse IgG1	130-093-502, 130-093-503, 130-093-504, 130-093-505, 130-093-506
CD49d-FITC, -PE, -APC, -Biotin, pure	MZ18-24A9, mouse IgG2b	130-093-283, 130-093-282, 130-093-281, 130-093-280, 130-093-279
Mouse cells		
Anti-FoxP3-PE, -APC	3G3, mouse IgG1	130-093-014, 130-093-013
FoxP3 Staining Buffer Set		130-093-142
CD4 -FITC, -PE, -APC	GK1.5, rat IgG2b	130-091-605, 130-091-607, 130-091-611
Anti-GITR-PE, -APC, pure, pure – functional grade	DTA-1, rat IgG2b	130-092-469, 130-092-470, 130-092-630, 130-092-655
CD25-PE, -APC, -Biotin	7D4, rat IgM	130-091-013, 130-093-734, 130-092-569
Isotype control antibodies		
Mouse IgG1-FITC, -PE, -APC, -VioBlue, - PerCP, -Biotin	IS5-21F5, mouse IgG1	130-092-213, 130-092-212, 130-092-214, 130-094-670, 130-094-968, 130-093-018
Mouse IgG2a-FITC, -PE, -APC, -VioBlue, -PerCP	S43.10, mouse IgG2a	130-091-837, 130-091-835, 130-091-836, 130-094-671, 130-094-967
Mouse IgG2b-FITC, -PE, -APC, -Biotin	IS6-11E5.11, mouse IgG2b	130-092-216, 130-092-215, 130-092-217, 130-092-466
Mouse IgM-FITC, -PE, -APC, -Biotin	IS5-20C4, mouse IgM	130-093-178, 130-093-177, 130-093-176, 130-093-175

Order information

Place your order by fax, phone, or online!

Products for the functional analysis or expansion of Treg cells

Product	Capacity/components	Order no.
Human		
Treg Suppression Inspector	2.5 mL	130-092-909
Treg Expansion Kit	2×2 mL	130-095-345
Treg Expansion Kit	2 mL	130-095-353
Mouse		
Treg Expansion Kit	2 mL	130-095-925

Molecular analysis of Treg cells

Product	Capacity/components	Order no.
mRNA isolation and cDNA synthesis		
µMACS One-step cDNA Starting Kit	20 reactions	130-091-989
µMACS One-step cDNA Kit	20 reactions	130-091-902
Cell type–specific total RNA		
Regulatory T Cell (CD4+CD25+) Total RNA, human	0.5 µg	130-093-936
For gene expression studies		
RNA extraction from human or animal cells (GEP)		160-001-563
SuperAmp Amplification Service		160-000-936
Agilent Whole Human Genome Microarray Service 8x60K, one-color		160-001-592
Agilent Whole Human Genome Microarray Service 8x60K, two-color		160-001-589
For microRNA expression studies		
RNA extraction from human or animal cells (miRNA analysis)		160-001-585
miRXplore Microarray Service		160-001-583
miRXplore Microarray Universal Reference Service		160-001-582
Agilent Human miRNA Microarray Service		160-001-598
For comparative genome hybridization studies		
Genomic DNA Extraction Service		160-000-963
Agilent Human Genome CGH Microarray 180K Service		160-001-576

References

More than 14,500 studies used Miltenyi Biotec products

CD4$^+$CD25$^+$ Regulatory T Cell Isolation Kit for human or mouse (p. 4)

1. Mougiakos *et al.* (2009) Naturally occurring regulatory T cells show reduced sensitivity toward oxidative stress–induced cell death. Blood 113: 3542–3545.
2. Schwaab *et al.* (2009) Clinical and immunologic effects of intranodal autologous tumor lysate-dendritic cell vaccine with aldesleukin (interleukin 2) and IFN-α2a therapy in metastatic renal cell carcinoma patients. Clin. Cancer Res. 15: 4986–4992.
3. Hong *et al.* (2006) CD4$^+$ regulatory T cell responses induced by T cell vaccination in patients with multiple sclerosis. Proc. Natl. Acad. Sci. USA 103: 5024–5029.
4. Nishikawa *et al.* (2006) Influence of CD4$^+$CD25$^+$ regulatory T cells on low/high-avidity CD4$^+$ T cells following peptide vaccination. J. Immunol. 176: 6340–6346.
5. Yang *et al.* (2006) Intratumoral CD4$^+$CD25$^+$ regulatory T-cell-mediated suppression of infiltrating CD4$^+$ T cells in B-cell non-Hodgkin lymphoma. Blood 107: 3639–3646.
6. Ou *et al.* (2004) T regulatory cells in atopic dermatitis and subversion of their activity by superantigens. J. Allergy Clin. Immunol. 113: 756–763.
7. Li *et al.* (2008) CD4$^+$CD25$^+$ Treg cells inhibit human memory γδ T cells to produce IFN-γ in response to M tuberculosis antigen ESAT-6. Blood 111: 5629–5636.
8. Schwarz *et al.* (2004) Ultraviolet radiation-induced regulatory T cells not only inhibit the induction but can suppress the effector phase of contact hypersensitivity. J. Immunol. 172: 7006–7013.
9. Gangi *et al.* (2005) IL-10–producing CD4$^+$CD25$^+$ regulatory T cells play a critical role in granulocyte-macrophage colony-stimulating factor-induced suppression of experimental autoimmune thyroiditis. J. Immunol. 174: 7006–7013.
10. Chauhan *et al.* (2009) Autoimmunity in dry eye is due to resistance of Th17 to Treg suppression. J. Immunol. 182: 1247–1252.
11. Turk *et al.* (2004) Concomitant tumor immunity to a poorly immunogenic melanoma is prevented by regulatory T cells. J. Exp. Med. 200: 771–782.
12. Kashiwada *et al.* (2006) Downstream of tyrosine kinases-1 and Src homology 2-containing inositol 5′-phosphatase are required for regulation of CD4$^+$CD25$^+$ T cell development. J. Immunol. 176: 3958–3965.

CD4$^+$CD25$^+$CD127$^{dim/-}$ Regulatory T Cell Isolation Kit, human (p. 5)

13. Liu *et al.* (2006) CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4$^+$ T reg cells. J. Exp. Med. 203: 1701–1711.
14. Seddiki *et al.* (2006) Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. J. Exp. Med. 203: 1693–1700.
15. Tran *et al.* (2009) Regulatory T cells allows for their purification from expansion cultures receptor type I/II (CD121a/CD121b) on activated human FOXP3$^+$ selective expression of latency-associated peptide (LAP) and IL-1. Blood 113: 5125–5133.
16. Stockis *et al.* (2009) Membrane protein GARP is a receptor for latent TGF-β on the surface of activated human Treg. Eur. J. Immunol. 39: 3315–3322.

CD4$^+$CD25$^+$CD45RA$^+$ Regulatory T Cell Isolation Kit, human (p. 5)

17. Valmori *et al.* (2005) A peripheral circulating compartment of natural naive CD4 Tregs. J. Clin. Invest. 115: 1953-1962.
18. Hoffmann *et al.* (2006) Only the CD45RA$^+$ subpopulation of CD4$^+$CD25^{high} T cells gives rise to homogeneous regulatory T-cell lines upon *in vitro* expansion. Blood 108: 4260–4267.

CD25$^+$CD49d$^-$ Regulatory T Cell Isolation Kit, human (p. 5)

19. Kleinewietfeld *et al.* (2009) CD49d provides access to "untouched" human Foxp3$^+$ Treg free of contaminating effector cells. Blood 113: 827–836.

Tr1 cell analysis (p. 6)

20. Veldmann *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.
21. Akdis *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.
22. Meiler *et al.* (2008) *In vivo* switch to IL-10–secreting T regulatory cells in high dose allergen exposure J. Exp. Med. 205: 2887–2898.
23. Dong *et al.* (2007) IL-10 is excluded from the functional cytokine memory of human CD4$^+$ memory T lymphocytes. J. Immunol. 179: 2389–2396.
24. Battaglia *et al.* (2006) Rapamycin and interleukin-10 treatment induces T regulatory type 1 cells that mediate antigen-specific transplantation tolerance. Diabetes 55: 40–49.

Detection of Treg cell surface markers (p. 8)

25. Liu *et al.* (2006) CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4$^+$ T reg cells. J. Exp. Med. 203: 1701–1711.
26. Seddiki *et al.* (2006) Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. J. Exp. Med. 203: 1693–1700.

Treg Suppression Inspector (p. 9)

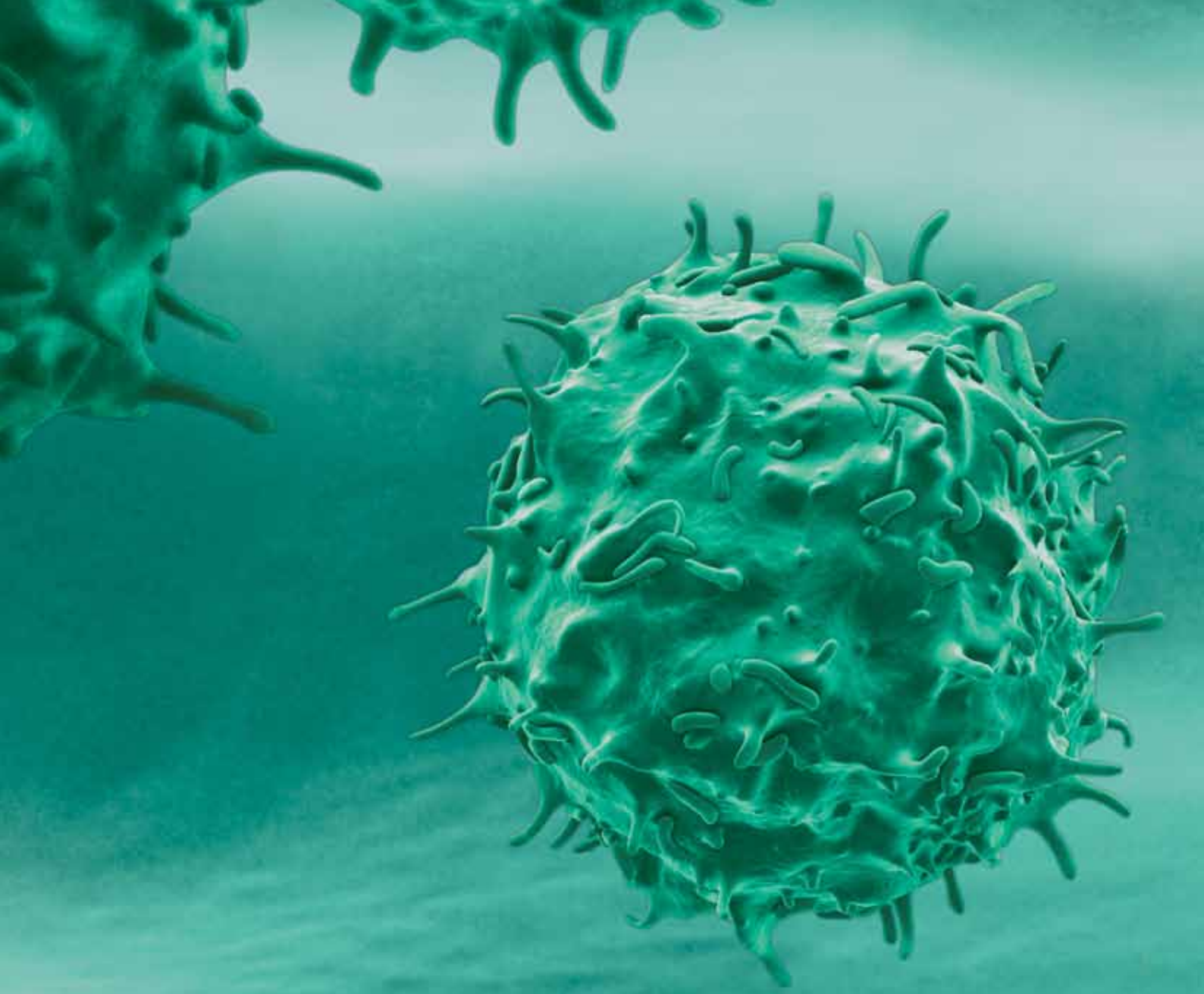
27. Kleinewietfeld *et al.* (2009) CD49d provides access to "untouched" human Foxp3$^+$ Treg free of contaminating effector cells. Blood 113: 827–836.
28. Mougiakos *et al.* (2009) Naturally occurring regulatory T cells show reduced sensitivity toward oxidative stress–induced cell death. Blood 113: 3542–3545.
29. Schwaab *et al.* (2009) Clinical and immunologic effects of intranodal autologous tumor lysate-dendritic cell vaccine with aldesleukin (interleukin 2) and IFN-α2a therapy in metastatic renal cell carcinoma patients. Clin. Cancer Res. 15: 4986–4992.

µMACS™ One-step cDNA Technology (p. 10)

30. Fecci *et al.* (2006) Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. Cancer Res. 66: 3294–3302.
31. Learn *et al.* (2006) Profiling of CD4$^+$, CD8$^+$, and CD4$^+$CD25$^+$CD45RO$^+$FoxP3$^+$ T cells in patients with malignant glioma reveals differential expression of the immunologic transcriptome compared with T cells from healthy volunteers. Clin. Cancer Res. 12: 7306–7315.

microRNA expression analysis (p. 10)

32. Lodish *et al.* (2008) Micromanagement of the immune system by microRNAs. Nature Rev. Immunol. 8: 120–130.
33. Bartel (2009) MicroRNAs: target recognition and regulatory functions. Cell 136: 215–233.
34. Landgraf *et al.* (2007) A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 129: 1401–1414.



Miltenyi Biotec

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

USA/Canada
Miltenyi Biotec Inc.
2303 Lindbergh Street
Auburn, CA 95602, USA
Phone 800 FOR MACS
Phone +1 530 888 8871
Fax +1 530 888 8925
macs@miltenyibiotec.com

Australia
Miltenyi Biotec
Australia Pty. Ltd.
Unit 16A, 2 Eden Park Drive
North Ryde, NSW 2113,
Australia
Phone +61 2 8877 7400
Fax +61 2 9889 5044
macs@miltenyibiotec.com.au

Benelux
Miltenyi Biotec B.V.
Schipholweg 68 H, 2316 Leiden
The Netherlands
macs@miltenyibiotec.nl

Customer service Netherlands
Phone 0800 4020120
Fax 0800 4020100
Customer service Belgium
Phone 0800 94016
Fax 0800 99626
Customer service Luxembourg
Phone 800 24971
Fax 800 24984

China
Miltenyi Biotec GmbH
Shanghai Office
Rm. 2309–2310,
No. 319 Xianxia Rd.
Shanghai 200051, P.R. China
Phone +86 21 62351005
Fax +86 21 62350953
macs@miltenyibiotec.com.cn

France
Miltenyi Biotec SAS
10 rue Mercoeur
75011 Paris, France
Phone +33 1 56 98 16 16
Fax +33 1 56 98 16 17
macs@miltenyibiotec.fr

Italy
Miltenyi Biotec S.r.l.
Via Persicetana, 2/D
40012 Calderara di Reno (BO)
Italy
Phone +39 051 6 460 411
Fax +39 051 6 460 499
macs@miltenyibiotec.it

Japan
Miltenyi Biotec K.K.
Nittsu-Eitai Building 5F
16-10 Fuyuki, Koto-ku,
Tokyo 135-0041, Japan
Phone +81 3 5646 8910
Fax +81 3 5646 8911
macs@miltenyibiotec.jp

Singapore
Miltenyi Biotec
Asia Pacific Pte Ltd.
100 Beach Road
#28-06 to 28-08 Shaw Tower
Singapore 189702
Phone +65 6238 8183
Fax +65 6238 0302
macs@miltenyibiotec.com.sg

Spain
Miltenyi Biotec S.L.
C/Luis Buñuel 2
Ciudad de la Imagen
28223 Pozuelo de Alarcón
(Madrid), Spain
Phone +34 91 512 12 90
Fax +34 91 512 12 91
macs@miltenyibiotec.es

United Kingdom
Miltenyi Biotec Ltd.
Almac House, Church Lane
Bisley, Surrey GU24 9DR, UK
Phone +44 1483 799 800
Fax +44 1483 799 811
macs@miltenyibiotec.co.uk

130-093-700.04

www.miltenyibiotec.com

Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.