

Regulatory T cell research

Unique kits for cell isolation

Harmonized cell culture and expansion tools

Convenient functional assay tools

Reliable flow cytometry analysis

Introduction

The complete workflow for regulatory T cell research

Regulatory T (Treg) cells are a subpopulation of T cells, which are characterized by their immune suppressive function. They are essential to maintain tolerance to self-antigens and to suppress overwhelming reactions against pathogens. Due to their outstanding role within the immune system, Treg cells are of paramount interest for immunology research and with our broad portfolio of products we aim to provide a complete workflow solution for the investigation of Treg cells. We support you during all steps of your experiments.

- Broad Treg cell product portfolio to fit your research needs
- Optimized tools for your Treg cell separation, expansion and suppression assays
- Standardized cell culture medium and cytokines up to GMP grade for optimal Treg culture conditions



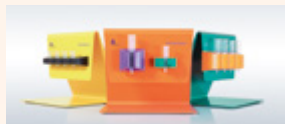
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- Viable cell suspensions through gentle tissue dissociation
- Fast and reliable, user-independent results

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MACS® Technology

- Fast and efficient isolation of Treg cells from human whole blood or PBMCs
- Isolation of mouse and non-human primate Treg cells

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TexMACS™ Medium

- Cell culture products perfectly suited for *in vitro* expansion and characterization of Treg cells

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REAffinity™ Antibodies

- Easy-to-use Treg Detection Kits
- Broad portfolio of recombinantly engineered REAffinity Antibodies for the characterization of Treg cell subsets

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Regulatory T cell workflow

Research

Clinical



gentleMACS™ Dissociators

- Tissue Dissociation Kits

SAMPLE PREPARATION



Leukapheresis



MACSxpress® Separator

- MACSxpress® Treg Isolation Kit, human



MACS® MultiStand

- CD4⁺CD25⁺ Regulatory T Cell Isolation Kit, human
- CD4⁺CD25⁺CD127^{dim/-} Regulatory T Cell Isolation Kit, human
- CD4⁺CD25⁺CD45RA Regulatory T Cell Isolation Kit, human



autoMACS® pro Separator

- CD25⁺CD49⁺ Regulatory T Cell Isolation Kit, human
- CD4⁺CD25⁺ Regulatory T Cell Isolation Kit, mouse
- CD4⁺CD25⁺ Regulatory T Cell Isolation Kit, non-human primate

CELL SEPARATION



CliniMACS® Systems

- CliniMACS® CD25 Reagent*
- CliniMACS CD8 Reagent*
- CliniMACS CD19 Reagent*
- CliniMACS Anti-Biotin Reagent*



MACSQuant® Tyto™

- MACS® GMP Fluorescent Antibodies*:
 - CD25-PE-Biotin
 - CD4-VioBlue
 - CD127-APC
 - CD45RA-FITC
 - CD25-PE
 - CD4-PerCP



MACS Cell Culture

- TexMACS™ Medium, research grade
- Treg Expansion Kit, human
- Treg Expansion Kit, mouse
- Treg Suppression Inspector, human

CELL CULTURE



MACS® GMP Products

- TexMACS™ GMP Medium*
- MACS® GMP ExpAct Treg Kit*
- MACS GMP Recombinant Human IL-2*
- MACS GMP Rapamycin*
- MACS GMP Cell Expansion Bags*
- MACS GMP Cell Differentiation Bags*



REAffinity™ Antibodies

- Treg Detection Kit (CD4/CD25/CD127), human
- Treg Detection Kit (CD4/CD25/FoxP3), human
- Treg Detection Kit (CD4/CD25/FoxP3), mouse
- NEW:** REAffinity™ Antibodies for regulatory T Cell detection and characterization

FLOW CYTOMETRY

- Treg Detection Kit (CD4/CD25/CD127), human (**RUO**)
- Treg Detection Kit (CD4/CD25/FoxP3), human (**RUO**)
- NEW:** REAffinity™ Antibodies (**RUO**) for regulatory T cell detection and characterization



MACSQuant® Portfolio

RUO=Research Use Only

* For complete regulatory and legal notices please refer to the back page of the brochure.

Start smart

The secret to success lies in your starting material

gentleMACS™ Dissociators

Experience the gentleMACS™ Portfolio of versatile benchtop tissue dissociators that can process up to eight samples in parallel. They come with programmable protocol settings and optional temperature regulation to ensure standardized tissue dissociation.

- **Gentle:** viable single-cell suspensions with preserved epitopes
- **Reproducible:** pre-defined programs for standardized tissue processing
- **Fast:** processing of up to eight samples in parallel with the gentleMACS™ Octo Dissociator
- **Safe:** sample processing in a closed and sterile system

Various Dissociation Kits and instrument settings as well as comprehensive technical support are available for many tissue types. However, please note that to preserve CD25 expression on Treg cells, some dissociation kit protocols have to be modified. Please contact your local sales representative or our technical support team for more information.



Figure 1: The gentleMACS Dissociators for efficient tissue dissociation. Our versatile and reliable benchtop dissociators allow processing of up to 8 tissue samples in parallel.

gentleMACS™ Tubes

Tubes for the gentleMACS™ Dissociators, precision engineered to guarantee highest quality tissue dissociation or homogenization.

- **gentleMACS C Tubes:** use the purple C Tubes for high yields of viable single cells
- **gentleMACS M Tubes:** choose the orange M Tubes to thoroughly homogenize cells for, e.g., molecular analyses



Figure 2: Let the tubes do the work for you. gentleMACS C and M Tubes provide effective dissociation or homogenization of any tissue sample.

Select the best

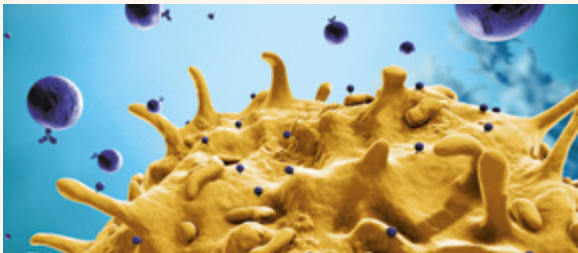
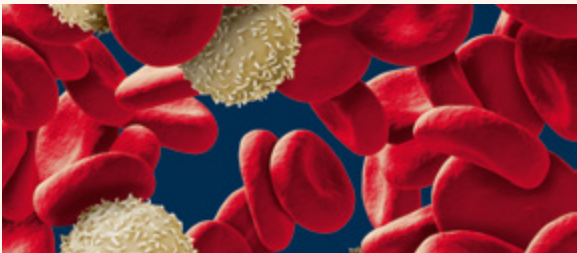
Choose the optimal solution for your experiment

MACS® Technology

One portfolio for all your T cell isolation needs: With the MACS® Cell Separation portfolio, you enjoy the freedom to choose the cell isolation option that's best for your specific requirements. Our proven magnetic cell separation technology is continuously expanding to offer new and innovative options across basic and clinical research.

Unsurpassed possibilities for the isolation of T cells:

- Nano-sized beads (column-based technology with MACS MicroBeads, Cell Isolation Kits, and Whole Blood MicroBeads) for highest purity and recovery
- Micro-sized beads (column-free MACSxpress® Technology) for maximum convenience and fastest isolations

	Nano-sized beads	Micro-sized beads
		
Benefits	• The most comprehensive cell isolation technology available • Applicable to virtually any species or sample material • Limitless range of use – from basic research to clinical applications	• Cell isolation from whole blood within 20 minutes • No density gradient centrifugation • High reproducibility due to minimal sample handling
Components	• MACS Columns • MACS Separators • MACS MicroBeads	• MACSxpress® Separator • MACSxpress Beads
Starting material	• PBMCs • Whole blood • Bone marrow • Single-cell suspensions	• Whole blood
	MACS MicroBead Technology	MACSxpress Technology

MACS® Columns

The most gentle way to isolate your target cells

The MACS® Column advantage

At the heart of MACS® MicroBead Technology is the MACS Column (fig. 3), the vessel for our patented ferromagnetic matrix for cell separation. Designed and manufactured to deliver maximum purity and yield of viable cells, the MACS Column and matrix provide the solution for any sample type or volume.

- **Minimal cell labeling** with nano-sized MicroBeads is sufficient to isolate cells effectively.
- **Gentle to cells** as they can freely flow through the column.

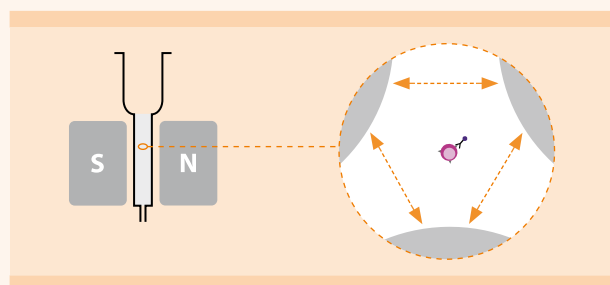


Figure 3: The MACS MicroBead Technology. The column matrix consists of spheres that are between 250 and 500 μm in size, depending on the column type. Within the matrix, the space between the spheres is 20 times the size of lymphocytes, allowing unhindered flow of cells through the column.

Explore the possibilities

We have matrix-based separation strategies for all sample sizes and types. We offer a variety of options, ranging from manual systems for low throughput experiments to fully automated systems that can process an increased number of samples (fig. 4).

Discuss your specific application with a local representative for a customized recommendation.

 Manual MACS® Separators	Manual cell separation of up to eight samples in parallel that is easy to use and simple to incorporate into any lab.		Fully automated cell separation for increased sample throughput and reproducibility.	 autoMACS® Pro Separator
 MultiMACS™ Cell24 Separator Plus	Semi-automated separation of up to 24 samples in parallel for fast, high-quality cell separations compatible with your cell separation strategy.		Integrated cell separation solutions to simplify complex workflows with robotic assistance.	 Robotic integration

Figure 4: MACS Cell Separation options at a glance. From MACS Manual Separators to fully robotic integration of MACS Instruments into your liquid handling system, the MACS Column and matrix enable fast and effective cell separation to support your research.

The convenient route to separation

Isolate Treg cells with high purity and excellent recovery

What's your starting material?

Our Regulatory T Cell Isolation Kits offer a convenient way to isolate functional CD4⁺CD25⁺ cells as well as other Treg subpopulations (fig. 5). The isolation of Treg cells by MACS[®] Technology yields positive and negative fractions of outstanding purity that are directly available for downstream applications, e.g., *in vitro* suppression assays.

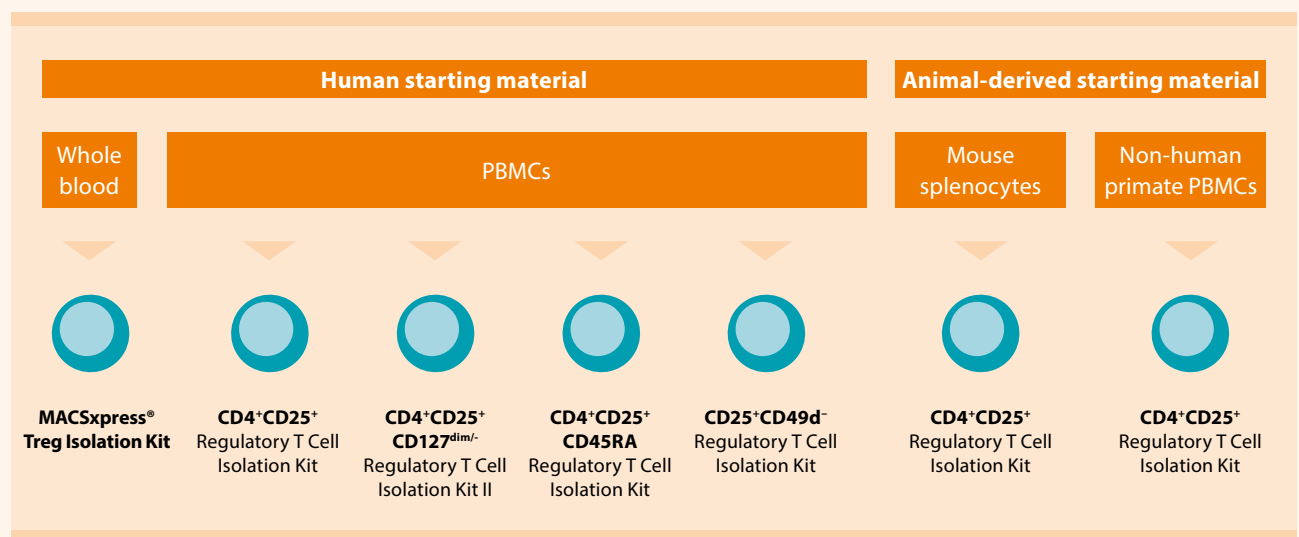


Figure 5: Pick your kit! Depending on your starting material and your Treg cell subset of interest, we provide a broad range of different Regulatory T Cell Isolation Kits to fulfill your needs.

Isolation of human Treg cells directly from whole blood

MACSxpress® Treg Isolation Kit, human

Our MACSxpress® Treg Isolation Kit has been developed for the isolation of CD4⁺CD25⁺ Treg cells from up to 30 mL of freshly drawn anticoagulated whole blood and offers several advantages:

- **Fast:** from whole blood to Treg cells in 30 minutes (fig. 6)
- **Convenient:** five simple steps without density gradient centrifugation (fig. 7)
- **Effective:** maximum recovery of Treg cells

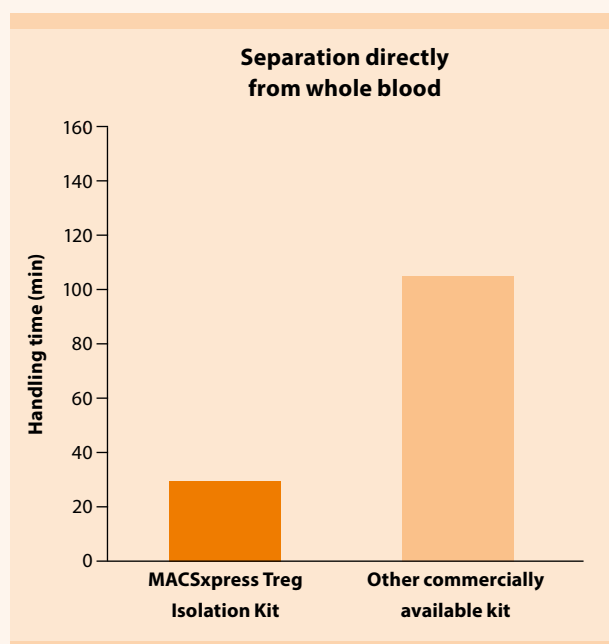


Figure 6: Save time with the MACSxpress Technology. Comparison of the MACSxpress cell separation strategy to another commercially available kit using magnetic cell separation to isolate Treg cells from whole blood shows that the handling time when using the MACSxpress Treg isolation Kit, human is greatly reduced. Only 30 min after starting the cell separation process the enriched Treg cells are ready for functional downstream assays without any further washing/preparation steps.

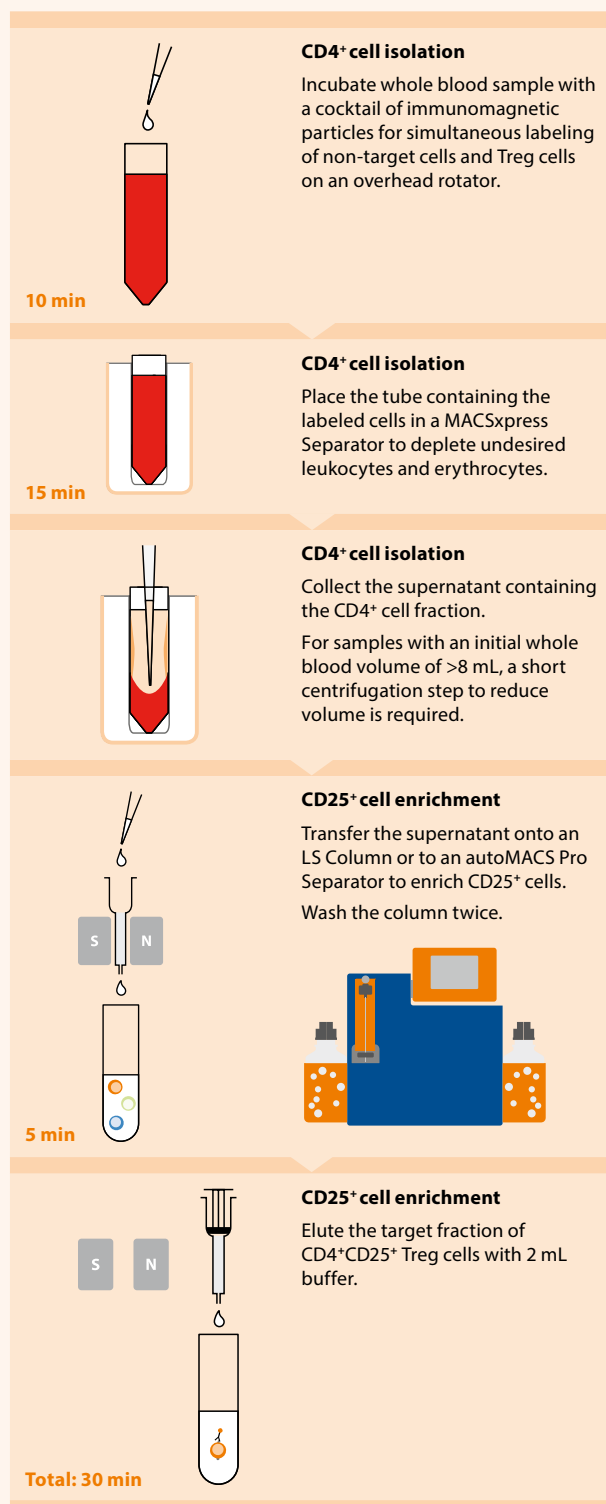


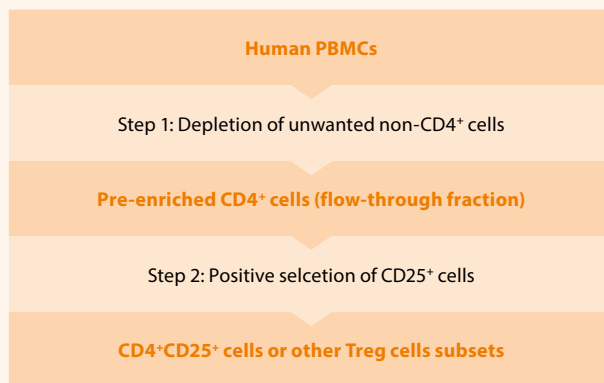
Figure 7: Fast and easy isolation of Treg cells. Isolation of CD4⁺CD25⁺ Treg cells straight from whole blood.

Often copied but never matched

Isolation of Treg cells and subsets with our Regulatory T Cell Isolation Kits

Isolation of human Treg subsets from PBMCs

We provide several Regulatory T Cell Isolation Kits for the isolation of different Treg cell subsets from PBMCs. The isolation is generally performed in a two-step procedure:



CD4⁺CD25⁺ Regulatory T Cell Isolation Kit, human

- For high purity, high recovery, and high quality
- Simultaneous isolation of CD4⁺CD25⁺ and CD4⁺CD25⁻, perfectly suited for *in vitro* suppression assays using the Treg Suppression Inspector, for example
- Isolation of $\sim 6 \times 10^5$ CD4⁺CD25⁺Treg cells/ 10^8 PBMCs

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

- For highest purity of FoxP3-positive cells
- Purity of up to 96% among WBC
- Isolation of 4×10^5 CD4⁺CD25⁺CD127^{dim/-} Treg cells/ 10^8 PBMCs

CD25⁺CD49d⁻ Regulatory T Cell Isolation Kit, human

- For isolation of Treg cells, depleted of CD25⁺CD49⁺ effector T cells
- Treg cell populations with the highest suppressive activity
- Isolation of $\sim 5 \times 10^5$ CD25⁺CD49d⁻ Treg cells/ 10^8 PBMCs

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

- For isolation of naive Treg cells
- Optimal Treg cell population for long-term *in vitro* expansion
- Isolation of $\sim 1.5 \times 10^5$ naive Treg cells/ 10^8 PBMCs

Isolation of mouse CD4⁺CD25⁺ Treg cells

Isolate CD4⁺CD25⁺ Treg cells from single-cell suspensions of mouse spleen and lymph nodes now even faster. The isolation is performed in a two-step procedure which allows downstream application of both, CD4⁺CD25⁺ Treg cells and CD4⁺CD25⁻ responder T cells. *In vitro* suppression assays and adoptive transfer, respectively, are just two possible examples for downstream applications.

CD4⁺CD25⁺ Regulatory T Cell Isolation Kit, mouse

- Simultaneous isolation of CD4⁺CD25⁺ Treg and CD4⁺CD25⁻ cells for, e.g., *in vitro* suppression assays
- Purity of up to 95%
- Isolation of 1.6×10^6 Treg cells per 1×10^8 splenocytes

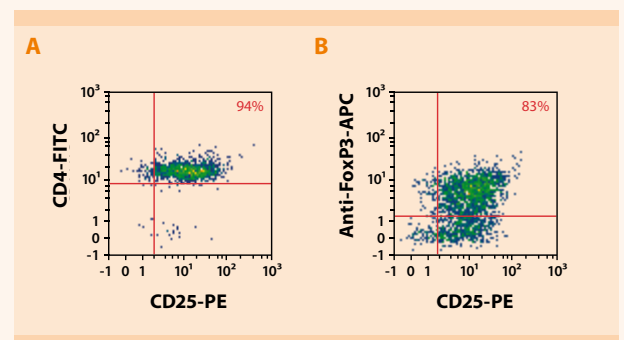


Figure 8: Isolation of mouse Treg cells using the CD4⁺CD25⁺ Regulatory T Cell Isolation Kit, mouse. Treg cells were isolated from mouse splenocytes using the CD4⁺CD25⁺ Regulatory T Cell Isolation Kit, mouse. The cells were fluorescently stained with CD4-FITC (A) or Anti-FoxP3-APC (B) and analyzed by flow cytometry. (Please note that the cells have already been labeled with CD25-PE during isolation).

Isolation of non-human primate CD4⁺CD25⁺ Treg cells

Isolate CD4⁺CD25⁺ Treg cells from rhesus monkey PBMCs with excellent purity and recovery. The isolated cells are the perfect starting material for your downstream applications including phenotypic and functional analyses.

CD4⁺CD25⁺ Regulatory T Cell Isolation Kit, non-human primate

- Purity of up to 92%
- Isolation of up to 9x10³ Treg cells per 1x10⁷ PBMCs
- Designed for isolation of Treg cells from rhesus monkey (*Macaca mulatta*)
- Cross-reactivity with cynomolgus monkey (*Macaca fascicularis*)

Special protocol: Isolation of CD4⁺CD25⁺CD127^{dim/-} Tregs from non-human primate PBMCs

With 2 simple modifications, you can use our human CD4⁺CD25⁺CD127^{dim/-} Regulatory T cell Isolation Kit II for the isolation of highly pure and functional non-human primate CD4⁺CD25⁺CD127^{dim/-} Treg cells.

Download this special protocol from our website at www.miltenyibiotec.com/SP-Treg or contact your local sales representative for further information.

Products for the isolation of Treg cells

Product	Capacity	Order no.
MACSxpress® Treg Isolation Kit, human	3x30 mL whole blood	130-109-557
MACSxpress CD4 T Cell Isolation Kit, human	3x30mL whole blood	130-098-195
Whole Blood CD4 MicroBeads, human	40mL whole blood	130-090-877
MACSxpress Starting Kit with MACSxpress Separator, MACSmix™ Tube Rotator, and one MACSxpress Isolation Kit of choice		130-101-319
MACSxpress® Separator		130-098-308
CD4 ⁺ CD25 ⁺ Regulatory T Cell Isolation Kit, human	1x10 ⁹ total cells	130-091-301
CD4 ⁺ CD25 ⁺ CD127 ^{dim/-} Regulatory T Cell Isolation Kit II, human	1x10 ⁹ total cells	130-094-775
CD4 ⁺ CD25 ⁺ CD45RA ⁺ Regulatory T Cell Isolation Kit, human	2x10 ⁹ total cells	130-093-631
CD25 ⁺ CD49d ⁻ Regulatory T Cell Isolation Kit, human	1x10 ⁹ total cells	130-094-551
MACS MultiStand		130-042-303
MidiMACS separator		130-042-302
LS Columns	25 pieces	130-042-401
LD Columns	25 pieces	130-042-901
MS Columns	25 pieces	130-042-201
CD25 MicroBeads II, human	1x10 ⁹ total cells	130-092-983
CD127 MicroBead Kit, human	1x10 ⁹ total cells	130-094-945
CD4 ⁺ CD25 ⁺ Regulatory T Cell Isolation Kit, mouse	1x10 ⁹ total cells	130-091-041
CD4 ⁺ CD25 ⁺ Regulatory T Cell Isolation Kit, non-human primate	1x10 ⁹ total cells	130-092-984

Skip the Ficoll gradient step and be fast and efficient:

Use our Whole Blood CD4 MicroBeads, human (positive isolation) or our MACSxpress CD4 T Cell Isolation Kit, human (untouched isolation) for pre-enrichment before flow sorting.

Ideal culture conditions give ideal results

Treg cell culture and expansion tools

The smart way to cultivate, expand, and analyze your Treg cells

Activation, expansion, and stimulation of Treg cells can be challenging. However, now you can trust our cell culture tools that are optimized for Treg cell culture. The combination of TexMACS™ Medium, MACS® Cytokines, Treg cell expansion tools, and the Treg Suppression Inspector are perfectly suited for *in vitro* culture and characterization of Treg cells.

TexMACS™ Medium, research grade

TexMACS™ Medium, research grade, is an optimized animal component-free cell culture medium. It was developed for the cultivation and expansion of T and Treg cells, enabling reproducible application in human and mouse cell culture.

Treg Suppression Inspector, human

The Treg Suppression Inspector, human has been developed for the functional characterization of human Treg cells by means of an *in vitro* suppression assay. It is based on MACSiBead Particles coupled to anti-CD2, anti-CD3 and anti-CD28 antibodies, which provide polyclonal stimuli to T cells. The Treg Suppression Inspector maintains an ideal stimulative environment for suppression assays: Responder T (Tresp) cells are stimulated while remaining susceptible to the suppression by Treg cells. Tregs cells remain hypoproliferative during the assay, which can be analyzed by ³H-Thymidine incorporation or by determining the dilution of a cell tracker reagent (fig. 9).

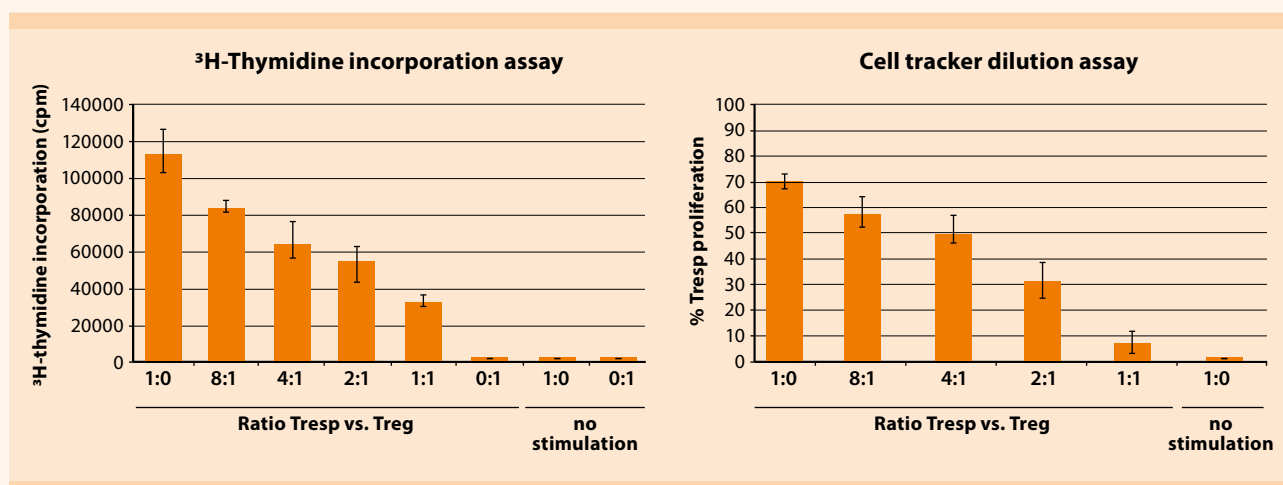


Figure 9: Treg suppression assay using the Treg Suppression Inspector, human. Treg and Tresp cells were co-cultured at different ratios in the presence or absence (no stimulation) of the Treg Suppression Inspector. Tresp cell proliferation was assessed by ³H-thymidin incorporation (left) added for 16 hours after 5 days of culture or by a cell tracker dilution (right) of the prior stained responder T cells after 5 days of co-culture.

Optimal expansion of your isolated Treg cells

Our Treg Expansion Kits are based on cell-sized MACSiBead™ Particles loaded with CD3 and CD28 antibodies. Optimized to perfection, a careful balance of the stimuli enables reliable Treg cell expansion with sustained FoxP3 expression. The MACSiMAG™ Separator ensures removal of MACSiBead Particles after expansion so your downstream experiments will not be affected.

Treg Expansion Kit, human

This kit preserves FoxP3 expression on expanded Treg cells that were previously isolated from human blood or PBMCs with one of our Regulatory T Cell Isolation Kits, human (fig. 10).

Treg Expansion Kit, mouse

This kit was especially developed for the efficient expansion of murine Treg cells. The kit sustains FoxP3 expression on expanded Treg cells that were previously isolated with the CD4⁺CD25⁺ Regulatory T Cell Isolation Kit, mouse.

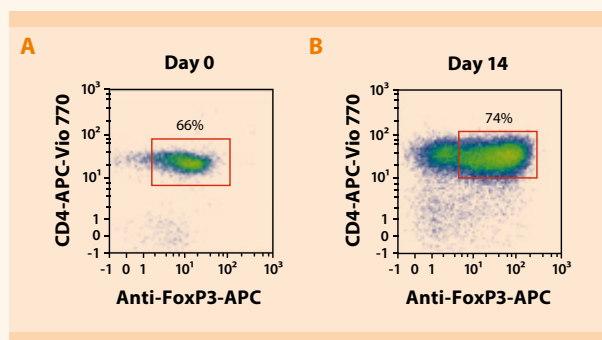


Figure 10: Expansion of Treg cells using the Treg Expansion Kit, human. Treg cells were isolated from whole blood using the MACSxpress Treg Isolation Kit, human and expanded with the Treg Expansion Kit, human according to the protocol for 14 days. Treg cells were analyzed for their expression of FoxP3 before (A) and after (B) expansion.

Products for cultivation and functional analysis of Treg cells

Product	Capacity	Order no.
TexMACS™ Medium, research grade	3x30 mL whole blood	130-097-196
Treg Suppression Inspector, human	2.5 mL	130-092-909
Treg Expansion Kit, human	2 mL	130-095-345
	2 x 2 mL	130-095-353
Treg Expansion Kit, mouse	2 mL	130-095-925
MACSiMAG™ Separator	Components: MACSiMAG Separator Tube rack for tubes from 0.5 mL to 5 mL in size	130-092-168
Human IL-2 IS, research grade Source: E. coli	10µg	130-097-742
	50µg	130-097-743
Human IL-2 IS, premium grade Source: E. coli	10µg	130-097-744
	50µg	130-097-745
	200µg	130-097-746
	1000µg	130-097-748
Mouse IL-2, research grade Source: E. coli	5µg	130-094-054
	20µg	130-094-055
	100µg	130-098-221
	1000µg	130-108-953

Identify your Treg subset of interest

Flow cytometry analysis of Treg cells

Solutions to power your cell analysis

When optimizing an experiment from beginning to end, complementary flow analysis tools lead you one step closer to reliable and reproducible results. The analysis tools of Miltenyi Biotec's Treg cell portfolio consist of a huge variety of MACS Antibodies for Treg-specific markers (fig. 11 and 12) as well as optimized ready-to-use Treg Detection Kits (mouse and human).

Treg Detection Kits

The Treg Detection Kits are optimized antibody kits with pre-titrated antibodies that ensure a reliable flow cytometric analysis of Treg cells. The Detection Kits have been developed as a simple one-reagent solution for the identification of human or mouse Treg cells.

REAffinity™ Antibodies

Most of our flow cytometry antibodies for Treg subset analysis are recombinantly generated antibodies that are engineered to lack any background binding. These recombinant antibodies, called REAffinity™ Antibodies, are available as conjugates to a variety of fluorochromes to address your multicolor flow needs – FITC, VioBright™ FITC, PE, APC, VioBlue®, VioGreen™, PE-Vio® 770, APC-Vio 770, PerCP-Vio 700.

Treg Detection Kits for the flow cytometric analysis of mouse and human Treg cells

Product	Capacity	Order no.
Anti-FoxP3-PE, human and mouse (Clone Isotype: 3G3)	30 tests	130-098-119
	100 tests	130-093-014
Anti-FoxP3-APC, human and mouse (Clone Isotype: 3G3)	30 tests	130-098-121
	100 tests	130-093-013
Treg Detection Kit (CD4/CD25/CD127), human	25 tests	130-096-076
	100 tests	130-096-082
Treg Detection Kit (CD4/CD25/FoxP3) (APC), human	100 tests	130-094-158
Treg Detection Kit (CD4/CD25/FoxP3) (PE), human	100 tests	130-094-163
Treg Detection Kit (CD4/CD25/FoxP3) (PE), mouse	100 tests	130-094-165
Treg Detection Kit (CD4/CD25/FoxP3) (APC), mouse	100 tests	130-094-164

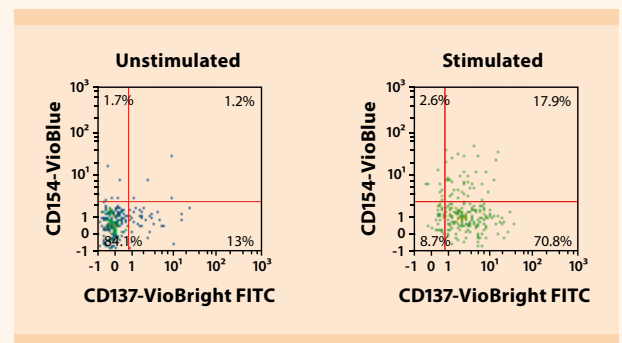


Figure 11: Flow cytometric analysis of human Treg cell activation marker expression. Human Treg cells were isolated from PBMCs by MACS using the CD4⁺CD25⁺CD127^{dim/-} Treg cell Isolation Kit II, human and either left unstimulated or stimulated with PMA/Ionomycin. After 16 hours, cells were stained with Viability 405/520 Fixable Dye, CD4-APC-Vio 770, Anti-FoxP3-APC, CD154-VioBlue and CD137-VioBright FITC. Data shown are gated on viable CD4⁺FoxP3⁺ lymphocytes.

Exemplary Treg flow analysis using our REAfinity™ Antibodies

Marker expression of Treg cell subsets. The combination of surface and intracellular markers enables the discrimination of different human Treg subsets

Treg subset	Surface marker	Intracellular marker	Population
Freshly isolated Tregs	CD4 ⁺ CD25 ⁺ CD127 ^{dim/-}	FoxP3, Helios	P1
Naive Tregs	CD4 ⁺ CD25 ⁺ CD127 ^{dim/-} CD45RA ⁺	FoxP3, Helios	P2
Memory Tregs	CD4 ⁺ CD25 ⁺ CD127 ^{dim/-} CD45RO ⁺	FoxP3, Helios, Ki-67 (partially)	P3

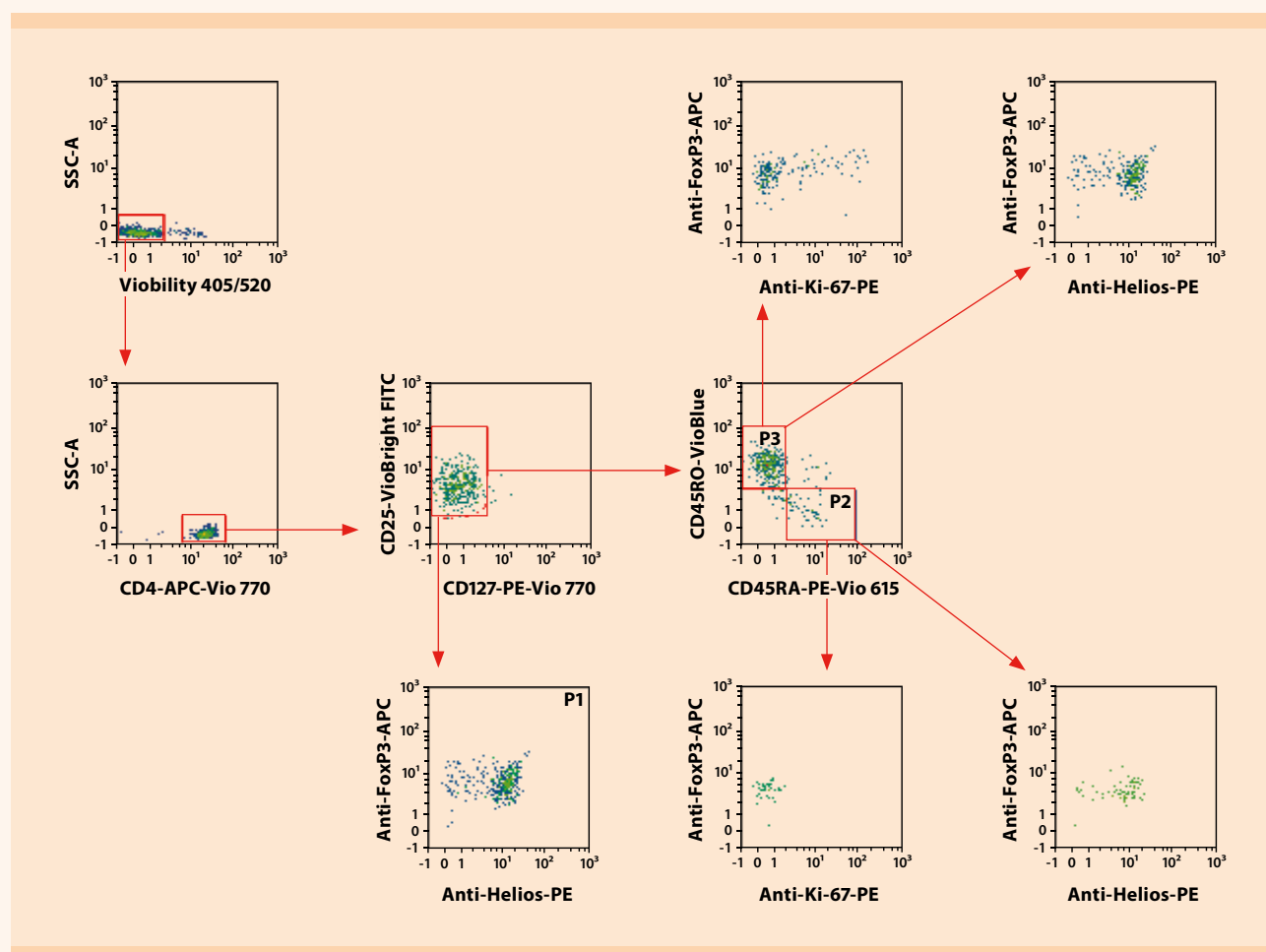


Figure 12: Gating strategy for identification of different Treg cells subsets. Human Treg cells were isolated from PBMCs by MACS® using the CD4⁺CD25⁺CD127^{dim/-} Regulatory T cell Isolation Kit II, human. Viable CD4⁺ cells were identified using the Viability 405/520 Fixable Dye and CD4-APC-Vio 770. Treg cells were stained with CD25-VioBright FITC, CD127-PE-Vio 770, Anti-FoxP3-APC, Anti-Helios-PE, CD45RA-PE-Vio 615, CD45RO-VioBlue, and Anti-Ki-67-PE. The gating strategy depicted identifies the different Treg populations P1, P2 and P3.



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