MOUSE T CELL RESEARCH

Cell Isolation and Culture

STEMCELL™ TECHNOLOGIES

Scientists Helping Scientists™ | WWW.STEMCELL.COM

EasySep™

The Easy Choice for Simple Cell Isolation

EasySep™ is a powerful immunomagnetic cell isolation platform that combines the specificity of monoclonal antibodies with the simplicity of a column-free magnetic system, offering easy and fast isolation of highly purified cell populations that are immediately ready for a wide range of downstream applications. EasySep™ protocols are optimized to minimize hands-on time, making cell isolation simpler and more efficient. This versatile technology supports positive selection, negative selection, or cell depletion and can be used to isolate cells in as little as 8 minutes from a variety of species (e.g. human, mouse, non-human primate, and rat) and sample sources, including splenocytes, lymph nodes, peripheral blood mononuclear cells (PBMCs), whole blood, leukopaks, cord blood, and bone marrow.

Why Use EasySep™ to Isolate Mouse Immune Cells?

- Isolate cells in as little as 15 minutes, with a simple pour.
- Achieve up to 99% cell purities with high recoveries.
- Obtain viable, functional cells without the need for columns and washes.
- Isolate cells from virtually any sample source.
- Choose a method backed by 20+ years of published research.

Request a Sample

Try EasySep™ Column-Free Cell Isolation
stemcell.com/Try-EasySep

EasySep™ Magnets

	EasySep™ Magnet[1]	"The Big Easy" EasySep™ Magnet[2]	Easy 50 EasySep™ Magnet	Easy 250 EasySep™ Magnet	EasyEights™ EasySep™ Magnet	EasyPlate™ EasySep™ Magnet
						
Catalog #	18000	18001	18002	100-0821	18103	18102
Number of Samples	1	1	1	1	8 on each side = 16 total	96
Start Sample Cell Number Range[3]	0.1 - 2.5 x 10^8 cells per 5 mL tube	0.2 - 10 x 10^8 cells per 14 mL tube	0.5 - 20 x 10^8 cells per 50 mL tube	2.00 - 12.5 x 10^9 cells	0.125 - 2.0 x 10^8 cells per 5 mL tube 0.25 - 8.0 x 10^8 cells per 14 mL tube	0.025 - 0.2 x 10^8 cells per well
Collection Method	Pour off	Pour off	Pipette off	Pipette off	Pipette off	Pipette off

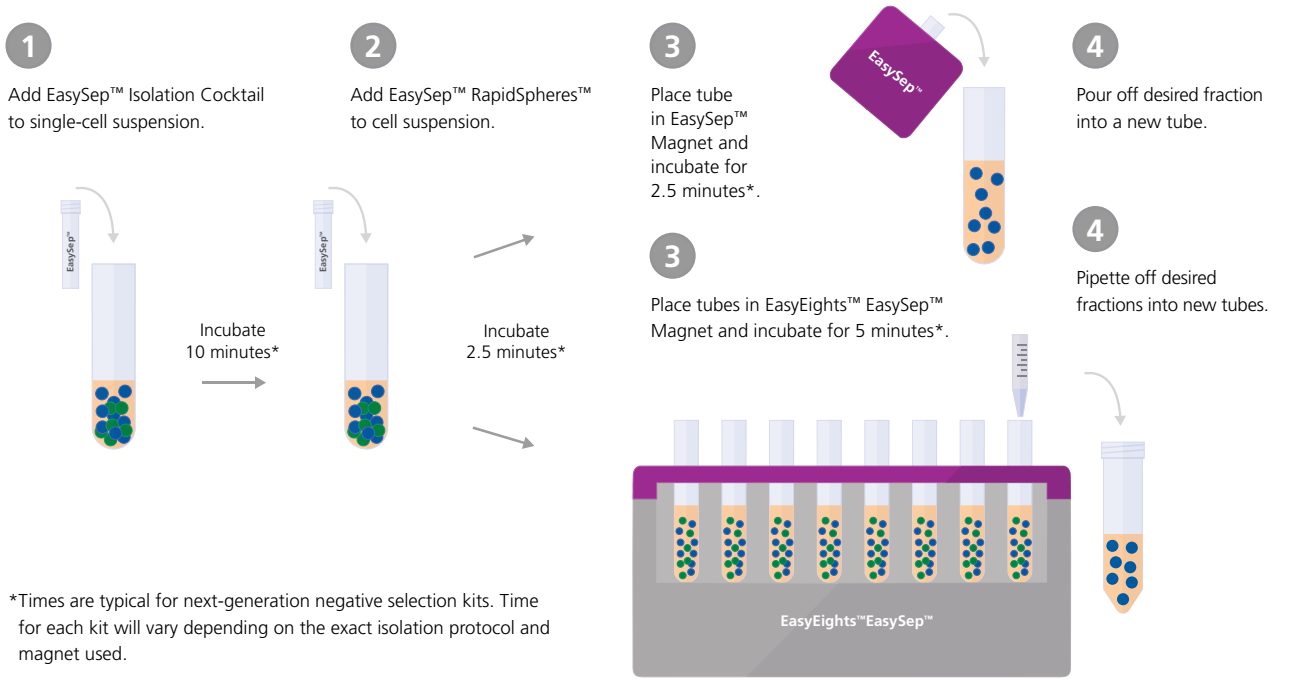
1. *Multiple EasySep™ magnets can be used together with the EasySep™ Multistand (Catalog #18010) for processing up to 4 samples simultaneously or with up to 6 EasySep™ EasyStands™ (Catalog #18130) for processing up to 6 samples.*
2. *Multiple "The Big Easy" EasySep™ magnets can be used together with the EasySep™ Multistand (Catalog #18010) for processing up to 4 samples simultaneously.*
3. *Minimum and maximum cell number range and volumes depend on the cell isolation kit, sample source, cell type being isolated, and flasks used during isolation.*

EasySep™ for Mouse Cells

Immunomagnetic Cell Isolation in As Little As 15 Minutes

EasySep™ kits are available for the isolation of mouse cells in as little as 15 minutes from a variety of sample sources, including spleen, bone marrow, lymph nodes, and whole blood. Our EasySep™ negative selection kits use biotinylated antibodies to target unwanted cells, while positive selection kits use an antibody complex to select cells of interest.

Typical EasySep™ Mouse Cell Isolation Protocol (Negative Selection)



*Times are typical for next-generation negative selection kits. Time for each kit will vary depending on the exact isolation protocol and magnet used.

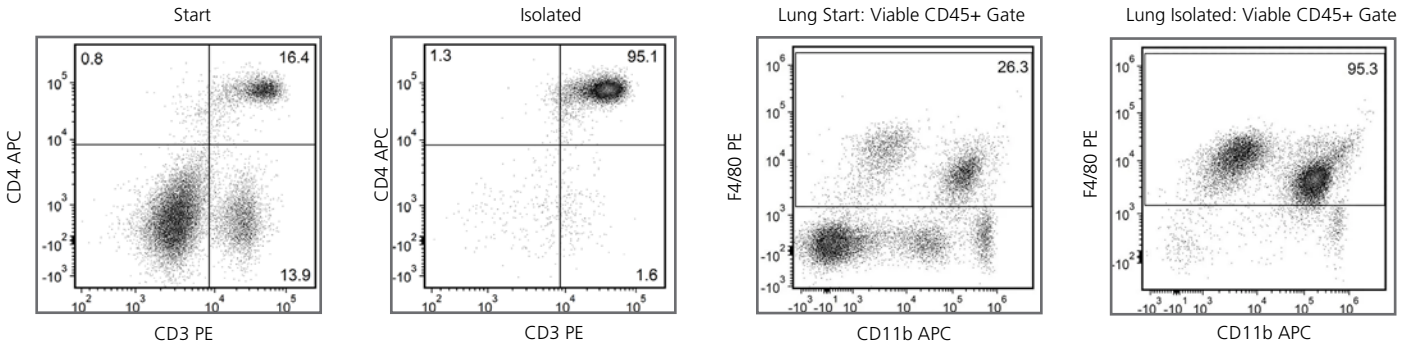


Figure 1. EasySep™ Mouse CD4⁺ T Cell Isolation Kit (Catalog #19852)

Starting with mouse splenocytes, the CD4⁺ T cell content (CD3⁺CD4⁺) of the isolated fraction is 95.4 ± 3% (mean ± SD using the purple EasySep™ magnet). In the above example, the purities of the start and isolated CD3⁺CD4⁺ fractions are 16.4% and 95.1%, respectively.

Figure 2. EasySep™ Mouse F4/80 Positive Selection Kit (Catalog #100-0659)

Starting with a naïve mouse lung single-cell suspension, the F4/80+ cell content of the isolated fraction is typically 94.3 ± 2.8% (mean ± SD using the purple EasySep™ magnet). In the above example, the purities of the start and final isolated fractions are 26.3% and 95.3%, respectively.

Mouse Cell Isolation Products

Mouse T Cell and Subset Isolation by Negative Selection

Cell Type	Source	Product	Purity[1]	For Processing	Compatible Staining Antibodies	Catalog #
T Cells	Spleen	EasySep™ Mouse T Cell Isolation Kit	96.6 ± 2.0%	1 x 10^9 cells	CD3e (Catalog #60015) CD90 (Catalog #60024)	19851 19851RF
Pan-Naïve T Cells	Spleen	EasySep™ Mouse Pan-Naïve T Cell Isolation Kit	90.0 - 97.0%	1 x 10^9 cells		19848 19848RF
CD4$^+$ T Cells	Spleen	EasySep™ Mouse CD4$^+$ T Cell Isolation Kit	95.4 ± 3%	1 x 10^9 cells	CD3e (Catalog #60015) CD4 (Catalog #60017)	19852 19852RF
Naïve CD4$^+$ T Cells	Spleen	EasySep™ Mouse Naïve CD4$^+$ T Cell Isolation Kit	90.0 - 95.0%	1 x 10^9 cells	CD4 (Catalog #60017) CD44 (Catalog #60068) CD62L (Catalog #60109)	19765 19765RF
Memory CD4$^+$ T Cells	Spleen	EasySep™ Mouse Memory CD4$^+$ T Cell Isolation Kit	78.0 - 96.0%	1 x 10^9 cells		19767 19767RF
CD8$^+$ T Cells	Spleen	EasySep™ Mouse CD8$^+$ T Cell Isolation Kit	94.4 ± 0.7%	1 x 10^9 cells	CD3e (Catalog #60015) CD8a (Catalog #60023)	19853 19853RF
Naïve CD8$^+$ T Cells	Spleen	EasySep™ Mouse Naïve CD8$^+$ T Cell Isolation Kit	92.0 - 98.0%	1 x 10^9 cells	CD8a (Catalog #60023) CD44 (Catalog #60068) CD62L (Catalog #60109)	19858 19858RF

Mouse T Cell and Subset Isolation by Positive Selection

Cell Type	Source	Product	Purity[1]	For Processing	Compatible Staining Antibodies	Catalog #
CD90.2+ (Thy 1.2) Cells	Spleen	EasySep™ Mouse CD90.2 Positive Selection Kit II	97.0 - 99.0%	2 x 10^9 cells	CD90.2	18951 18951RF
CD90.1+ (Thy 1.1) Cells	Spleen, Lymph Node, Whole Blood	EasySep™ Mouse CD90.1 Positive Selection Kit	93.5 ± 3.9%	2 x 10^9 cells	CD90 (Catalog #60024)	18958 18958RF
CD4+ Cells	Spleen	EasySep™ Mouse CD4 Positive Selection Kit II	94.8 ± 3.5 %	2 x 10^9 cells	CD3e (Catalog #60015) CD4 (Catalog #60029)	18952 18952RF
CD4+CD62L+ Cells	Spleen	EasySep™ Mouse CD4+CD62L+ T Cell Isolation Kit	92.0 - 97.0%	1 x 10^9 cells	CD4 (Catalog #60017) CD44 (Catalog #60068)	18765 18765RF
CD4+CD304+ Cells	Spleen	EasySep™ Release Mouse CD4+CD304+ Regulatory T Cell Isolation Kit	90.4 ± 3.0%	1 x 10^9 cells	CD4 (Catalog #60029) CD4 (Catalog #60017) FOXP3, clone FJK-16s	100-1570 100-1564
	Lymph Nodes		92.1 ± 0.7%			
CD4+CD304- Responder Cells	Spleen		94.1 ± 2.9%			
CD4+CD25+ Cells	Spleen	EasySep™ Mouse CD4+CD25+ Regulatory T Cell Isolation Kit II	70.0 - 93.0%	1 x 10^9 cells	CD4 (Catalog #60029) CD4 (Catalog #60017)	18783
CD25+ Cells	Spleen	EasySep™ Mouse CD25 Regulatory T Cell Positive Selection Kit	80.0 - 93.0%	1 x 10^9 cells	CD4 (Catalog #60017)	18782 18782RF
CD8a+ Cells	Spleen	EasySep™ Mouse CD8a Positive Selection Kit II	96.3 ± 1.4%	2 x 10^9 cells	CD3e (Catalog #60015) CD8a (Catalog #60023)	18953 18953RF

RoboSep™-S Reagent Kits (RF) contain an EasySep™ Selection Kit with RoboSep™ Buffer and 1 - 2 boxes of RoboSep™ Tip Racks.

1. *Purities shown as either a range or mean ± SD.*

Culture Cells with ImmunoCult™

Activate, Expand, Maintain, and Differentiate Immune Cells

Ensure optimal activation, expansion, and differentiation of immune cell subsets by using STEMCELL Technologies' ImmunoCult™ cell culture media and supplements. ImmunoCult™ products allow you to culture various cell types, including monocytes, T cells, NK cells, B cells, dendritic cells, and macrophages, under defined stimulatory conditions for consistent and reliable results.

ImmunoCult™ products are part of a wider and complete immunology workflow of STEMCELL products.

ImmunoCult™ Mouse T Cell Products

Product	Catalog #	Size
ImmunoCult™ Mouse T Cell Activator Kit	100-1572	3 x 1 mL
ImmunoCult™ -XF T Cell Expansion Medium	10981	500 mL
ImmunoCult™ Mouse Th1 Differentiation Supplement	10953	1 mL
ImmunoCult™ Mouse Th2 Differentiation Supplement	10955	1 mL
ImmunoCult™ Mouse Treg Differentiation Supplement	10957	1 mL

Why Use ImmunoCult™?

- Activate, expand, or differentiate immune cells in culture conditions optimized to promote high yield and frequency.
- Minimize variation by using serum-free culture conditions.
- Consistently achieve high yields of immune cells with the desired phenotype and function.
- Mix and match media, activators, and supplements to suit your specific research needs.

Wallchart

Frequencies & Percentages of Mouse Immune Cell Types
stemcell.com/MouseCellFreq

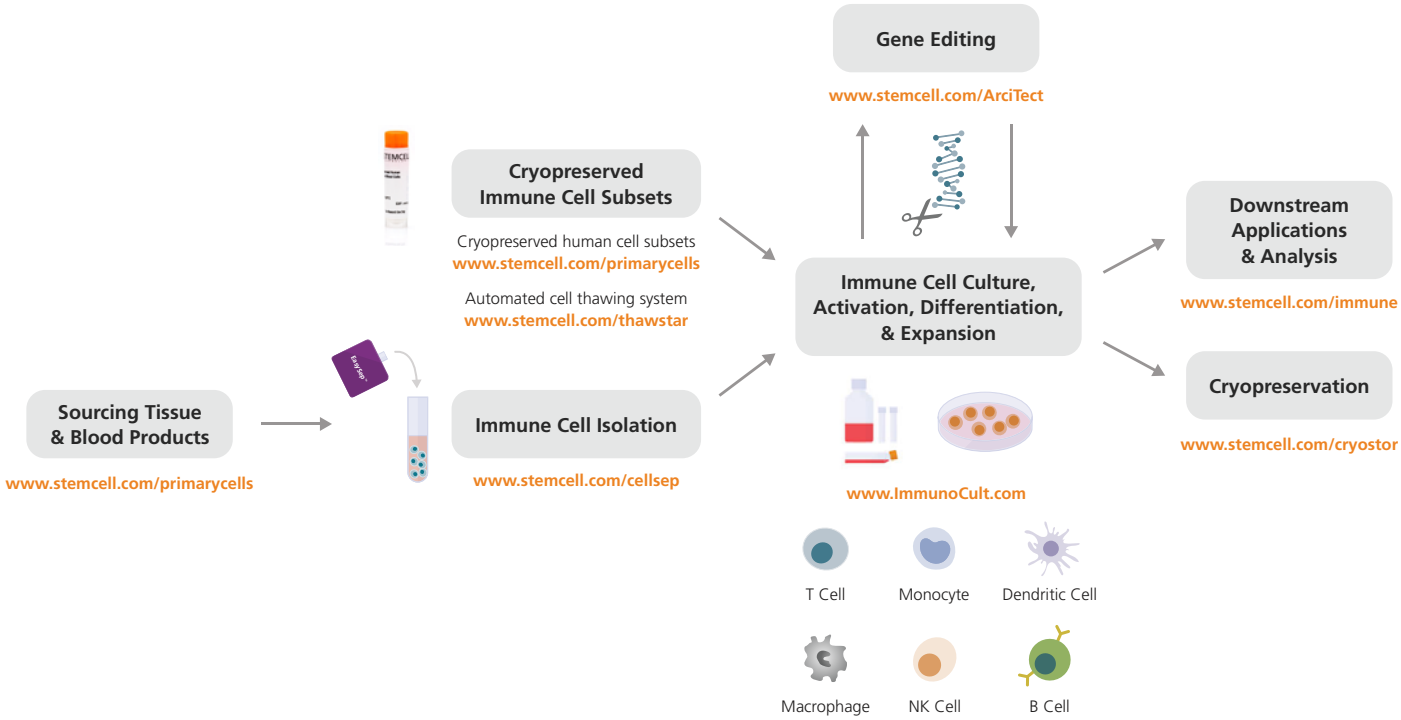


Figure 3. Products for Your Immune Cell Culture Workflow

ImmunoCult™ for Mouse T Cell Activation and Expansion

Activate mouse T cells with confidence using a single kit that allows for flexible design and activation by CD3/CD28 or CD3/CD28/CD2. The ImmunoCult™ Mouse T Cell Activator Kit provides a gentle activation stimulus that encourages high viability of activated T cells, which can then be further expanded in common mouse T cell growth media. For efficient isolation of mouse T cells, the ImmunoCult™ Mouse T Cell Activator Kit can be used with EasySep™ as a part of a complete T cell workflow.

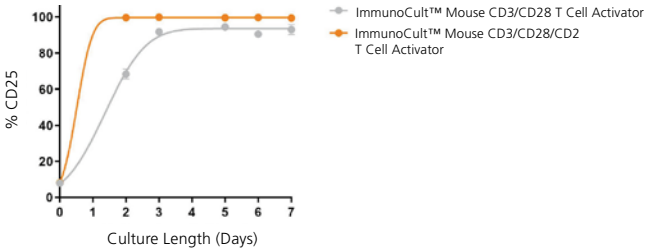


Figure 4. Activation of EasySep™-Isolated Mouse T Cells Stimulated with ImmunoCult™ Mouse T Cell Activator Kit

Mouse T cells were isolated using EasySep™ Mouse T Cell Isolation Kit, stimulated with ImmunoCult™ Mouse T Cell Activator Kit, and cultured in IMDM + FBS formulation. Following 3 days of culture, the mean ± SD frequency of CD25+ cells was 91.9 ± 5.1% (n = 11) or 99.9 ± 0.1% (n = 5) when stimulated with ImmunoCult™ Mouse CD3/CD28 T Cell Activator or ImmunoCult™ Mouse CD3/CD28/CD2 T Cell Activator, respectively. Stimulated mouse T cells maintained expression levels of CD25 throughout the 7-day culture period.

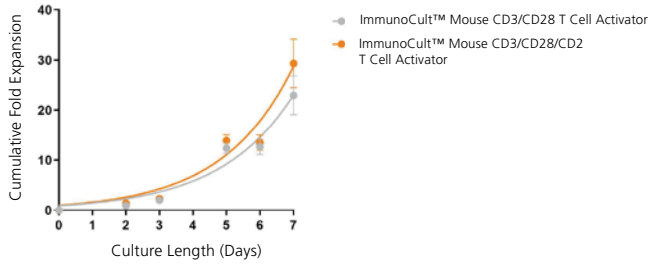


Figure 5. Robust Expansion of EasySep™-Isolated Mouse T Cells Can Be Achieved Following Stimulation with ImmunoCult™ Mouse T Cell Activator Kit

EasySep™-isolated mouse T cells were expanded with ImmunoCult™ Mouse T Cell Activator Kit in IMDM + FBS formulation over 7 days. The number of viable cells was assessed every 2 - 3 days, when fresh medium supplemented with IL-2 was added. No additional ImmunoCult™ Mouse T Cell Activator was added during the 7-day culture period. After 7 days in culture with ImmunoCult™ Mouse CD3/CD28 T Cell Activator or ImmunoCult™ Mouse CD3/CD28/CD2 T Cell Activator, stimulation resulted in a fold expansion of 23 ± 3.4 or 29.3 ± 4.8 (mean ± SEM, n = 6), respectively.

Why Use ImmunoCult™ Activation and Expansion Reagents?

- Achieve optimal activation without the use of magnetic beads, feeder cells, or antigens.
- Maintain functional T cells with high viability by using a gentle activation stimulus.
- Rely on highly stable, filter-sterilized, and soluble reagents that are compatible with common T cell growth medium.

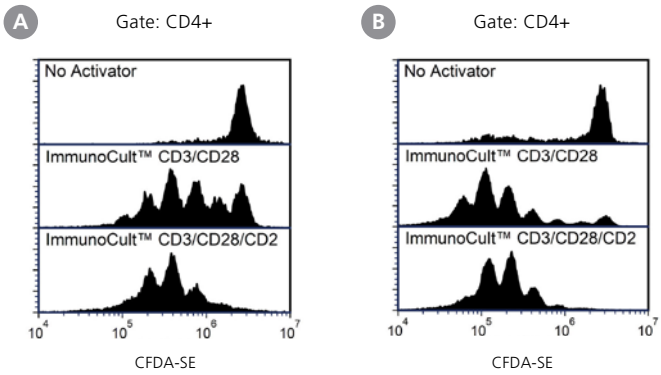


Figure 6. High Cell Proliferation Is Observed in EasySep™-Isolated T cells After Stimulation with ImmunoCult™ Mouse T Cell Activator

EasySep™-isolated mouse T cells were labeled with CFDA-SE (Catalog #75003), stimulated with ImmunoCult™ Mouse T Cell Activator Kit, and cultured in IMDM + FBS formulation. On Day 3, cells were harvested, stained with anti-mouse CD4 and CD8a antibodies, then measured by flow cytometry. Shown are CFDA-SE-labeled mouse T cells, gated on viable (A) CD4+ or (B) CD8a+ cells, cultured with no activator (top panel), with ImmunoCult™ Mouse CD3/CD28 T Cell Activator (middle panel), or with ImmunoCult™ Mouse CD3/CD28/CD2 T Cell Activator (bottom panel). Due to cell proliferation, the intensity of CFDA-SE signal is reduced by 50% for each cell division.

Protocol Video

How to Prepare a Single-Cell Suspension from Mouse Spleen

stemcell.com/Mouse-Sample-Processing

ImmunoCult™ for Mouse T Cell Differentiation

Differentiate mouse T cells with ImmunoCult™ supplements. These differentiation supplements contain recombinant mouse cytokines and blocking monoclonal antibodies formulated to promote the differentiation of spleen-derived mouse naïve CD4⁺ T cells into Th1, Th2, or regulatory T (Treg) cells. ImmunoCult™ differentiation supplements are intended for use with RPMI 1640 Medium containing fetal bovine serum and other additives, as well as anti-mouse CD3 and CD28 monoclonal antibodies as activating agents.

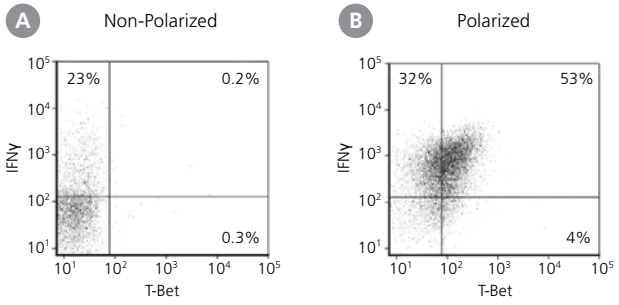


Figure 7. ImmunoCult™ Mouse Th1 Differentiation Supplement Produces CD4⁺IFNγ⁺T-Bet⁺ Cells Under Th1 Polarizing Conditions

Naïve CD4⁺ T cells were isolated from mouse splenocytes using the EasySep™ Mouse Naïve CD4⁺ T Cell Isolation Kit (Catalog #19765), activated with plate-bound anti-CD3 and soluble anti-CD28, and cultured in medium alone (non-polarized cultures) or medium supplemented with ImmunoCult™ Mouse Th1 Differentiation Supplement (polarized cultures) for 5 days. Cells were subsequently stimulated with PMA/Ionomycin, in conjunction with Brefeldin A treatment, and stained with anti-CD4, anti-IFNγ, anti-T-bet, and a viability dye, and analyzed by flow cytometry. Shown is the expression of IFNγ and T-bet, back-gated on viable CD4⁺ cells from (A) non-polarized or (B) polarized cultures. The mean proportion of CD4⁺IFNγ⁺T-Bet⁺ cells is significantly higher in cells cultured in ImmunoCult™ Mouse Th1 Differentiation Supplement (44 ± 6%) compared to non-polarized cells (2 ± 1%; $p < 0.001$; $n = 13$). Data from experimental groups were compared using a paired T-test.

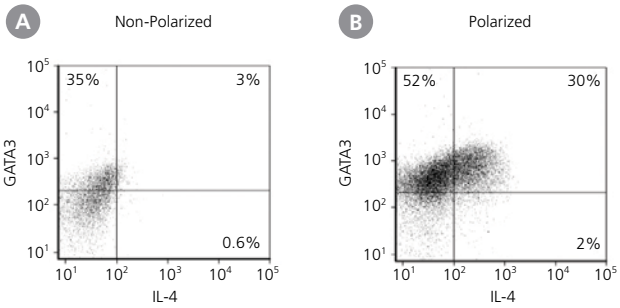


Figure 8. ImmunoCult™ Mouse Th2 Differentiation Supplement Produces CD4⁺GATA3⁺IL-4⁺ Cells Under Th2 Polarizing Conditions

Naïve CD4⁺ T cells were isolated from mouse splenocytes using the EasySep™ Mouse Naïve CD4⁺ T Cell Isolation Kit (Catalog #19765), activated with plate-bound anti-CD3 and soluble anti-CD28, and cultured in medium alone (non-polarized cultures) or in medium supplemented with ImmunoCult™ Mouse Th2 Differentiation Supplement (polarized cultures) for 6 days. Cells were subsequently stimulated with PMA/Ionomycin, in conjunction with monensin treatment, and stained with anti-CD4, anti-IL-4, anti-GATA3, and a viability dye, and analyzed by flow cytometry. Shown is the expression of GATA3 and IL-4 back-gated on viable CD4⁺ cells from (A) non-polarized or (B) polarized cultures. The mean proportion of CD4⁺IL-4⁺GATA3⁺ cells is significantly higher in cells cultured in ImmunoCult™ Mouse Th2 Differentiation Supplement (25 ± 3%) compared to non-polarized cells (4 ± 1%; $p < 0.001$; $n = 10$). Data from experimental groups were compared using a paired T-test.

Why Use ImmunoCult™ Differentiation Supplements?

- Induce Th1, Th2, or Treg cells from naïve CD4⁺ T cells isolated from mouse spleen.
- Add 100x concentrated supplements directly to the medium.
- Use cell culture reagents that are compatible with upstream EasySep™ cell isolation.

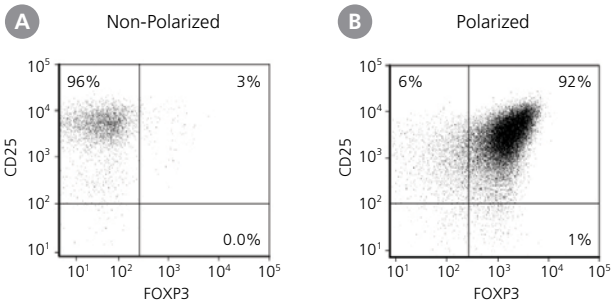


Figure 9. ImmunoCult™ Mouse Treg Differentiation Supplement Produces CD4⁺CD25⁺FOXP3⁺ Cells Under Treg Polarizing Conditions

Naïve CD4⁺ T cells were isolated from mouse splenocytes using the EasySep™ Mouse Naïve CD4⁺ T Cell Isolation Kit (Catalog #19765), activated with plate-bound anti-CD3 and soluble anti-CD28, and cultured in medium alone (non-polarized cultures) or in medium supplemented with ImmunoCult™ Mouse Treg Differentiation Supplement (polarized cultures) for 6 days. Cells were subsequently stained with anti-CD4, anti-CD25, anti-FOXP3, and a viability dye, and analyzed by flow cytometry. Shown is the expression of CD25 and FOXP3 back-gated on viable CD4⁺ cells from (A) non-polarized or (B) polarized cultures. The mean proportion of CD4⁺FOXP3⁺ cells is significantly higher in cells cultured in ImmunoCult™ Mouse Treg Differentiation Supplement (91 ± 2%) compared to non-polarized cells (2 ± 0.4%; $p < 0.001$; $n = 14$). Data from experimental groups were compared using a paired T-test.

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