

Technical Data Sheet

Human Regulatory T Cell Cocktail

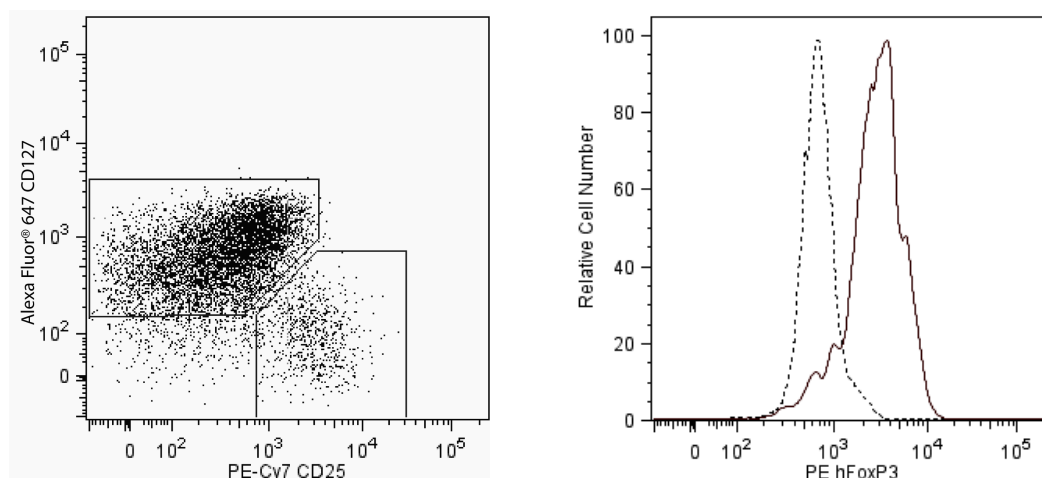
Product Information

Material Number:	560249
Size:	50 tests
Vol. per Test:	20 ul
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Cocktail Component	Clone	Isotype
FITC anti-Human CD4	SK3	mIgG1, κ
PE-Cy7 anti-Human CD25	2A3	mIgG1, κ
Alexa Fluor® 647 anti-Human CD127	HIL-7R-M21	mIgG1, κ

The Human Regulatory T cell cocktail is a three color reagent used to identify the natural T regulatory cell (nTreg) population. An expression pattern of CD4+CD25^{int}/brightCD127^{dim} closely correlates to the expression of the transcription factor Forkhead box P3 (FoxP3). FoxP3 is considered a specific marker to T regulatory cells (Tregs). The SK3 antibody recognizes CD4, an antigen expressed primarily on helper/inducer T lymphocytes, although this antigen is also present in low density on monocytes. The 2A3 antibody recognizes CD25, an antigen also known as the human low-affinity interleukin-2 receptor (IL-2R). The CD25 antigen is present on a subset of peripheral blood lymphocytes. Antigen density increases on phytohemagglutinin (PHA)-, concanavalin A (Con A)-, and CD3-activated T lymphocytes; T lymphocytes from mixed lymphocyte cultures; and human T-lymphocyte leukemia virus (HTLV)-infected T-lymphocyte leukemia lines. hIL-7R-M21 reacts with the 60 - 90 kDa glycoprotein, CD127. CD127 is also known as the IL-7 receptor alpha (IL-7Rα). The receptor is a heterodimer composed of the CD127 and the common gamma chain, shared by other cytokine receptors (IL-2R, IL-4R, IL-9R, and IL-15R). CD127 is expressed on thymocytes, T- and B-cell progenitors, mature T cells, and some lymphoid and myeloid cells.



Three-color analysis of the expression of CD4, CD25, and CD127 on peripheral blood mononuclear cells (PBMCs). PBMCs were stained with either an Isotype Control (Cat. No. 557872/555909; data not shown) or Human Regulatory T Cell Cocktail (Cat. No. 560249). The PBMCs were then fixed, lysed and permeabilized using BD Pharmingen Human FoxP3 Buffer Set (Cat. No. 560098) and stained with PE conjugated anti-human FoxP3 monoclonal antibody (Cat. No. 560082). During data analysis, lymphocytes were identified by light scatter profile and CD4 positive expression. The figure on the left represents the CD25 and CD127 expression profile of the CD4 positive cells. The panel on the right shows hFoxP3 expression on CD127^{dim}/CD25^{bright} T regulatory cells (solid line) and other T cells (dashed line). Flow cytometry was performed on a BD FACSCanto™.

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

The antibody was conjugated with PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed.

The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry

Tested During Development

Recommended Assay Procedure:

For an Application Note on how to isolate and characterize regulatory T cells, please refer to the following link:

<http://www.bdbiosciences.com/pdfs/whitePapers/23-9373-00.pdf>

Suggested Companion Products

Catalog Number	Name	Size	Clone
555899	Lysing Buffer	100 ml	(none)
557872	PE-Cy TM 7 Mouse IgG1 κ Isotype Control	100 tests	MOPC-21
560098	Human FoxP3 Buffer Set	100 tests	(none)
560082	PE Mouse anti-Human FoxP3	25 tests	259D/C7

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^6 cells in a 100- μ l experimental sample (a test).
2. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
5. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
6. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BDTM Stabilizing Fixative (Cat. No. 338036).
7. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

1. Knapp, W., Dorken, B. et al., *Leucocyte Typing IV*. New York: Oxford University Press; 1989. (Biology)
- Liu W, Putnam AL, Xu-Yu Z, Szot GL et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4⁺ T reg cells. *J Exp Med*. 2006; 203(7):1701-1711. (Biology)
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- Schlossman SF, Boumsell L, Gilks W, et al, ed. *Leucocyte Typing V: White Cell Differentiation Antigens*. New York: Oxford University Press; 1995. (Biology)
- Seddiki N, Santner-Nanan B, Martinson J et al. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *J Exp Med*. 2006; 203(7):1693-1700. (Biology)