

updates in excellence



news from eBioscience

vol. 5 no. 2

Tregs & Foxp3: the Suppression Phenomenon

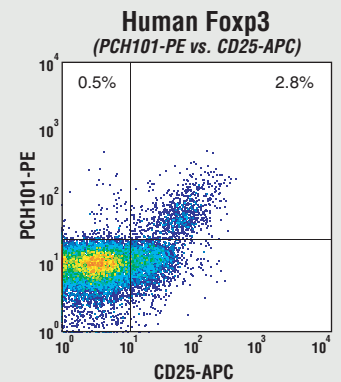
Foxp3 Antibodies Optimized for Flow Cytometry, IHC, WB

Until recently, definitive flow cytometric analysis of CD4+CD25+ regulatory T cells, expressing Foxp3, has been hindered due to lack of suitable Foxp3 antibodies. Using eBioscience Foxp3 antibodies PCH101, FJK-16s and NRRF-30, Foxp3 can now be identified at the single-cell level (see figures and table on page 2).

Since the discovery of regulatory suppressor cells by Gershon in 1970, great controversies have ensued. These cells have broad implications ranging from immune suppression to cancer. Consequently, the interest in the transcription factor Foxp3, identified by S. Sakaguchi (2003) as a hallmark of naturally arising CD4+CD25+ regulatory T cells (Tregs), has intensified.

Recently, one splice variant of Foxp3, lacking amino acids 71-105, has been reported in human T cells. This variant is expressed in CD8+ T cells from some donors, while both forms are present in CD4+CD25+ cells. The western blot data on page two reveals two bands in human tissue; presumably from each splice variant, while only a single band is detected in mouse. The presence of this splice variant has potentially complicated the complete characterization of Foxp3 expression.

Created by antibody experts in 1999, eBioscience is devoted to developing new tools to accelerate discoveries. Our quality-guaranteed products are available in trial and bulk sizes.



(continued on page 2)

Novel Mouse CCR7 Antibody: Defining Mouse Memory T Cell Subsets?

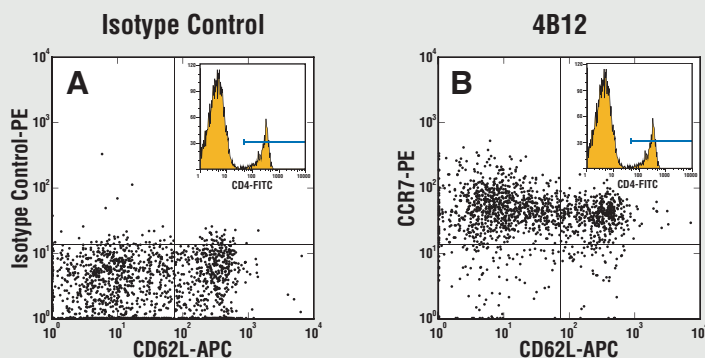
CCR7, also known as EBI-1 and CD197, is a chemokine receptor expressed on dendritic cells and lymphocytes. CCR7, via its interactions with CC-chemokine ligand 19 (also known as ELC) and/or CCL21 (also known as SLC) contributes to regulation of homing of T cells to lymph nodes through high endothelial venules.

In human, the cells defined functionally as central memory T cells (T_{CM}) generally are CCR7^{high} and CD62L^{high}, home to peripheral lymph nodes, lack immediate effector function, and can be stimulated to secrete IL-2. In contrast, effector memory T cells (T_{EM}) are CCR7^{low} and CD62L^{low}, are immediate effector cells, and secrete substantial amounts of IFN γ , IL-4 and IL-5, but not IL-2 upon stimulation. Until recently, most attempts to define CCR7 protein expression have been based on staining with the ligand, CCL19/ELC-Fc. However, it is unclear if such experiments yield direct evidence of CCR7 expression given the possible existence of other receptors capable of binding CCR7 ligands.

Definitive expression of CCR7 in the mouse is now facilitated by the availability of a new anti-mouse CCR7 monoclonal antibody, 4B12. Co-staining of splenocytes with 4B12 and anti-CD3 demonstrates that the majority of splenic T cells are 4B12 positive. Multicolor analysis of CD4, CD62L, and CCR7 (as shown in figure below) further defines the 4B12 staining pattern in mouse spleen. The staining profile of this monoclonal antibody 4B12 matches published reports of a polyclonal anti-CCR7 antibody (Bjorkdahl et al. 2003. Immunol. 110: 170)

Correlation of CCR7 expression with memory phenotype in mouse remains to be established. The availability of the 4B12 antibody in a variety of fluorochrome conjugate formats will enable for the first time a direct comparison of ELC-Fc and anti-CCR7 staining patterns in multicolor staining experiments to further define relative expression levels in different cell subsets, as has been shown for human cells.

Nature. 1999. 401(6754):708-12. Journal of Leukocyte Biology. 2004. 76: 472-476. Immunology. 2003. 110(2):170-9. Immunity. 2004. 21(2):279-88.



Mouse CCR7 expression, as detected by the 4B12 antibody. Mouse spleen cells were simultaneously stained with CD4-FITC (11-0041), CD62L-APC (17-0621), and CCR7-PE (12-1971). Viable cells were gated on the CD4+ population (inset). Dot plots present staining of isotype control-PE (A) or CCR7-PE (B) versus CD62L of the CD4-gated cells.

Clone	Formats (Cat. No.) - For reagent titre and sizes, see www.ebioscience.com
4B12	Purified (14-1971), Biotin (13-1971), PE (12-1971), APC (17-1971), PE-Cy7 (25-1971), Alexa*

* Alexa Fluor® is a registered trademark of and licensed under patents assigned to Molecular Probes Inc. for research use only. For specific Alexa Fluor® conjugates, please see www.ebioscience.com.

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Toll-Like Receptor Poster Inside

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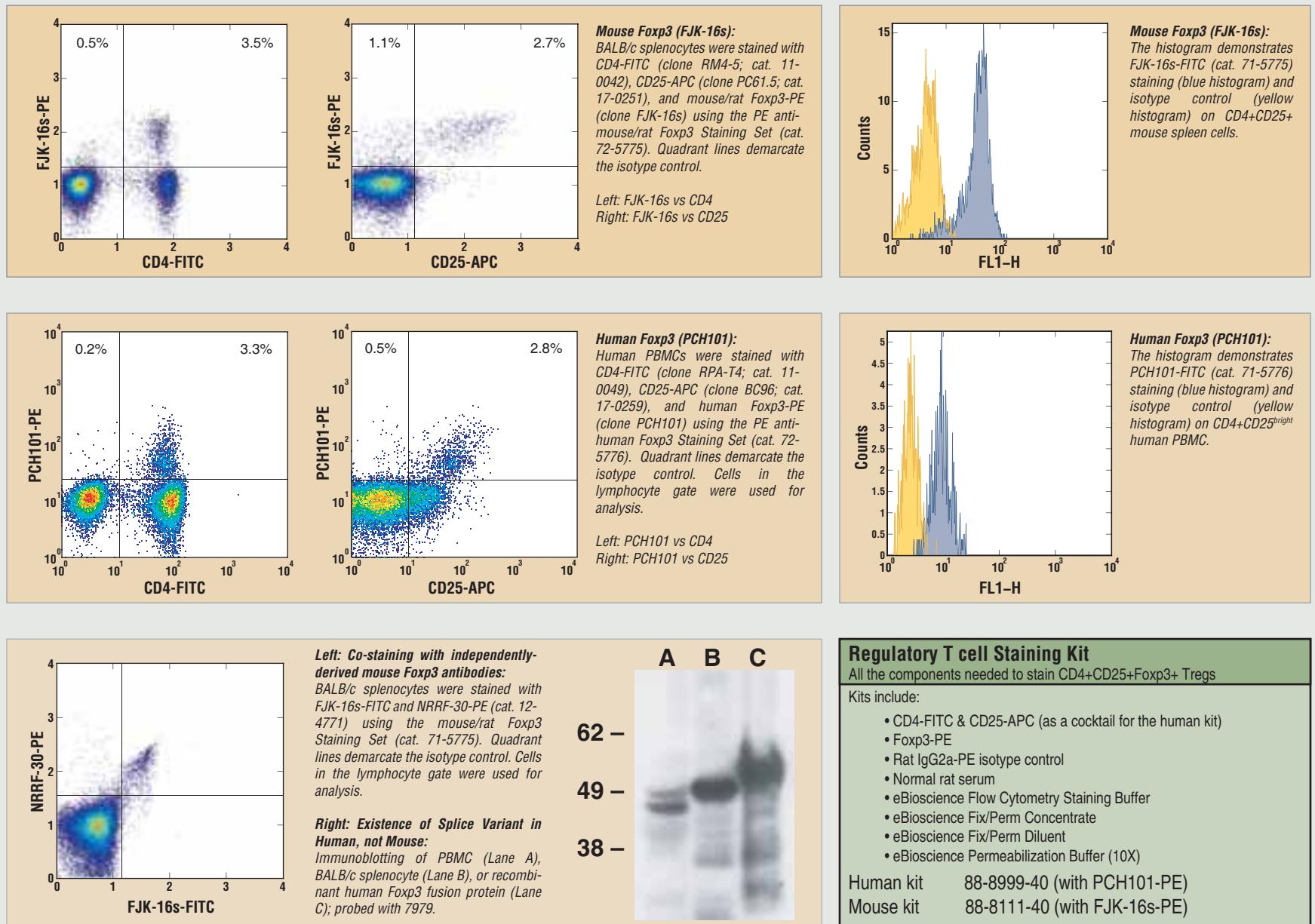
Design: Steven Lee

Tregs & Foxp3: the Suppression Phenomenon (continued from page 1)

Historically, characterizing the cellular expression profile of a new cellular antigen using only a single antibody may result in an incomplete picture of the protein expression profile. CD45 represents an antigen which was found to be alternatively spliced, which led to the need for isoform-specific antibodies for complete CD45 detection. This highlights the notion that for unequivocal immunophenotyping, several antibodies to different epitopes of an antigen are useful. To this end, eBioscience utilized protein deletion constructs to epitope-map a panel of Foxp3 antibodies. The anti-mouse Foxp3 antibodies, FJK-16s and NRRF-30 recognize different epitopes in the amino terminus, with the former recognizing the region corresponding to the human splice variant. These two antibodies identify the same population of lymphocytes, and co-staining experiments demonstrate 100% correlation (see figure below). These data suggest that these two independently-derived antibodies bind the same protein expressed in the same cells, and thereby confirm their specificity. Additionally, the intracellular staining data together with the western blot data confirm the absence, in mouse, of the reported human splice variant.

The expanding panel of antibodies reactive with distinct epitopes of Foxp3 is useful for investigating the complete Foxp3 protein expression profile at the single-cell level in both human and mouse, particularly with regards to its relationship to functionally-defined regulatory T cells. Because of the uniquely challenging nature of Foxp3 staining for flow cytometric analysis, it is critical to use the antibody-specific intracellular staining buffers and protocols for optimal results. Recent publications by Noelle et al (JI 2005. 175:1651) and other manuscripts in preparation, utilize these new tools to provide valuable insight into these powerful regulatory cells.

Science. 2003. 299:1057. Exp Hematol. 2004. 32:622. Blood. 2004. 104:3294. Int Immunol. 2004. 16:1055. Int Immunol. 2004. 16:1203. Immunity. 2005. 22: 329.



New Treg Research Reagents

Description	Clone	Application	Formats (Cat. No.)
Human Foxp3	PCH101	IC, WB	FITC Staining Set (71-5776), PE Staining Set (72-5776), APC Staining Set (77-5776), Staining Buffers (00-5523), Biotin (13-4776), Alexa*
Human/Mouse Foxp3	7979	WB	Purified (14-7979), FITC (11-7979)
Mouse/Rat Foxp3	FJK-16s	IC, WB	Purified (14-5773), Biotin (13-5773), FITC (11-5773), FITC Staining Set (71-5775), PE (12-5773), PE Staining Set (72-5775), APC (17-5773), APC Staining Set (77-5775), Staining Buffers (00-5523), Alexa*
Mouse Foxp3	NRRF-30	IC	PE (12-4771), Staining Buffer (00-5523)
Mouse GITR	DTA-1	FC, FA	Purified (14-5874), FG Purified (16-5874), Biotin (13-5874), FITC (11-5874), PE (12-5874), APC (17-5874), Alexa*
Mouse GITRL	YGL386	FC	Purified (14-5854), Biotin (13-5854), PE (12-5854), Alexa*

For proper Foxp3 detection, it is required that the eBioscience Foxp3 Staining Buffers and corresponding staining protocol be used with the appropriate antibody (NRRF-30, PCH101 and FJK-16s with 00-5523).

* Alexa Fluor® is a registered trademark of and licensed under patents assigned to Molecular Probes Inc. for research use only. For specific Alexa Fluor® conjugates, please see www.ebioscience.com.

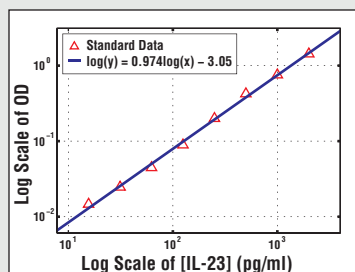
Mouse IL-23 ELISA

For specific measurement of IL-23 protein levels - without interference by IL-12

eBioscience is pleased to announce the availability of an ELISA for measurement of mouse IL-23 (p19p40). The Mouse IL-23 Ready-SET-Go! ELISA Set with high affinity binding ELISA plates provides the necessary antibodies, standards, buffers and diluents for performing quantitative, enzyme-linked immunosorbent assays (ELISA). The ELISA reagent set is specifically engineered for accurate and precise measurement of mouse IL-23 protein levels from samples including serum, plasma, and supernatants from cell cultures.

Parallelism: The assay demonstrates parallelism in measuring recombinant and native mouse IL-23 proteins with a standard curve range of 15 pg/ml to 2000 pg/ml and assay sensitivity below 15 pg/ml. Native mouse IL-23 was detected in supernatants from bone marrow-derived, LPS-activated dendritic cells.

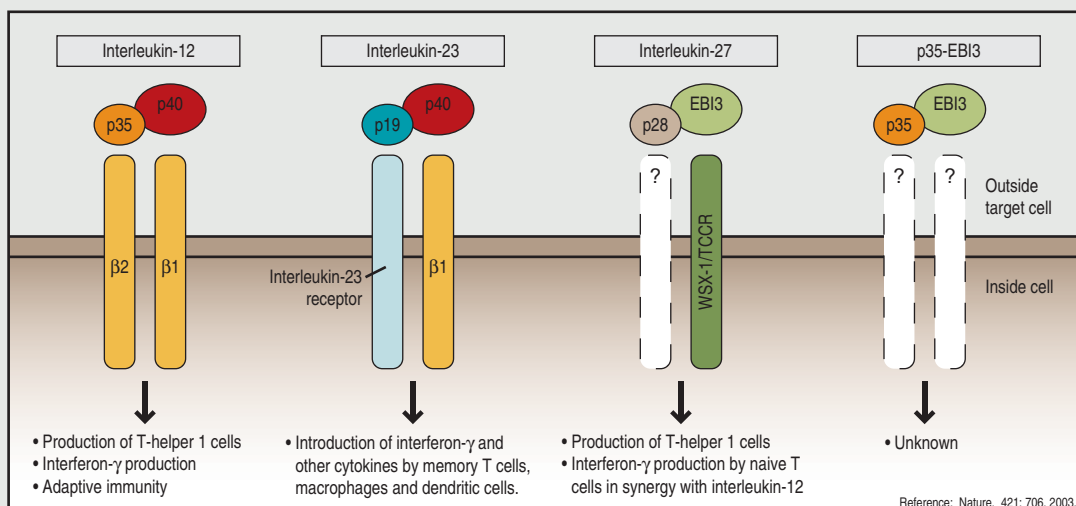
Specificity: The use of a p19-specific capture antibody and a p40-specific detection antibody renders this assay exquisitely specific for mouse IL-23. IL-12 p40 homodimer and IL-12 p70 were each run in the assay at 500 ng/ml with no interference or cross-reactivity observed. A panel of 20 unrelated cytokines was run in the IL-23 ELISA at 100 ng/ml with no cross reactivity observed; all values were at the limit of detection of the assay.



ELISA Ready-SET-Go! Sets and Kits

Description	Catalog No.	Format
Mouse IL-23 (p19/p40)	88-7234	Set
	88-7231	Kit*
Mouse IL-27 (p28/EBI3)	88-7274	Set
Mouse IL-12 (p70)	88-7121	Set
Human IL-23 (p19/p40)	88-7237	Set
	88-7239	Kit*

* Kits include pre-coated plates.



Reagent titre & data, guarantees, BestProtocols, formats, sizes & prices and other ordering information can be found on our website at www.ebioscience.com.

Neutralizing Anti-Mouse IL-23 p19 Monoclonal Antibody

For specific neutralization of mouse IL-23 with no effect on mouse IL-12 p70 bioactivity

Interleukin-23 is a novel, heterodimeric cytokine (p19/p40), sharing the p40 subunit of IL-12 (p35/p40), and sharing some bioactivities relating to induction of Th1-mediated immune responses. Mouse IL-23 induces strong proliferation of memory T cells (but not naïve T cells), whereas IL-12 has no effect on memory cells. Additionally, mouse IL-23 (but not IL-12) can activate mouse memory T cells to produce the proinflammatory cytokine IL-17. IL-23 has been reported to have anti-tumor and anti-metastatic activities, and has been found critical for development of autoimmune inflammation of the brain. IL-23 has been found to be secreted by activated dendritic cells. Unambiguous measurement of IL-23, without interference by the structurally- and functionally- related IL-12 p70 or p40 is critical for understanding the distinct roles of each of the IL-12 family members.

eBioscience's new G23-8 antibody reacts with the p19 subunit of mouse IL-23. The G23-8 antibody was generated from immunization with authentic, insect cell-expressed, recombinant mouse IL-23 heterodimer. The G23-8 antibody has been tested and found to specifically neutralize IL-23 bioactivity with no effect on IL-12 p70 bioactivity. For neutralization of IL-12 and IL-23 simultaneously, the C17.8 antibody (cat. 16-7123) is available.

FG Antibodies for Neutralization

Description	Clone	Catalog No.
Mouse IL-23 (p19)	G23-8	16-7232
Mouse IL-12/IL-23 (p40)	C17.8	16-7123
Human IL-23 (p19)	22A12	16-7238

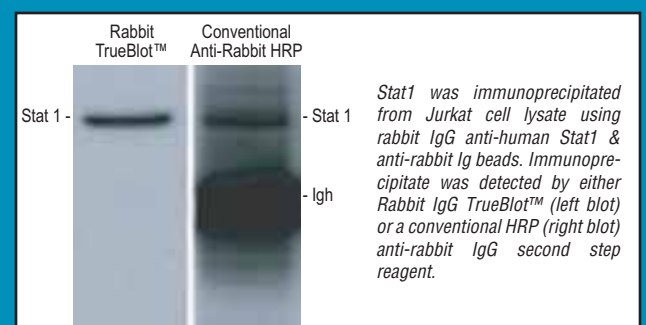
* Functional Grade (FG) Purified antibodies are azide-free.

IP/WB Discoveries Made Possible by TrueBlot™

For decades researchers performing IP/Westerns have been frustrated by contaminating heavy and light Ig bands that hinder the detection of their target bands. Because TrueBlot™ detects the blotting antibody and not the reduced IP antibody, new breakthroughs have been made by researchers worldwide.

Description	Formats (Cat. No.)
Goat	HRP IgG (18-8814), IP Beads (00-8844), Set (88-1488), WB Kit (88-8884)
Mouse	HRP IgG (18-8877), IP Beads (00-8811), Set (88-7788), WB Kit (88-8887)
Rabbit	HRP IgG (18-8816), IP Beads (00-8800), Set (88-1688), WB Kit (88-8886)
Rat	Coming Soon!

TrueBlot is a trademark of eBioscience, Inc.; patent pending.



It is easy to generate clean data with TrueBlot™ - simply substitute your conventional HRP blotting secondary with Goat TrueBlot™, Mouse TrueBlot™ or Rabbit TrueBlot™. TrueBlot™ has been used in the following publications:

Normal microRNA Maturation and Germ-Line Stem Cell Maintenance Requires Loquacious, a Double-Stranded RNA-Binding Domain Protein. *PLoS Biol.* 2005. 3:e236.
 Histone deacetylase 3 (HDAC3) activity is regulated by interaction with protein serine/threonine phosphatase 4. *Genes Dev.* 2005. 19:827.
 p25/Cdk5-mediated retinoblastoma phosphorylation is an early event in neuronal cell death. *J Cell Sci.* 2005. 118:1291.
 Requirement of the tyrosines at residues 258 and 270 of MAIR-1 in inhibitory effect on degranulation from basophilic leukemia RBL-2H3. *Int Immunol.* 2005. 17:65.
 Nephrin forms a complex with adherens junction proteins and CASK in podocytes and in Madin-Darby canine kidney cells expressing nephrin. *Am J Pathol.* 2004. 165:923.
 Silibinin causes cell cycle arrest and apoptosis in human bladder transitional cell carcinoma cells by regulating CDKI-CDK-cyclin cascade, and caspase 3 and PARP cleavages. *Carcinogenesis.* 2004. 25:1711.
 Regulation of Ca2+-induced permeability transition by Bcl-2 is antagonized by Drp1 and hFis1. *Molecular and Cellular Biochemistry.* 2005. 272:187.
 Focused proteomics: Monoclonal antibody-based isolation of the oxidative phosphorylation machinery and detection of phosphoproteins using a fluorescent phosphoprotein gel stain. *ELECTROPHORESIS* 2004. 25:2520