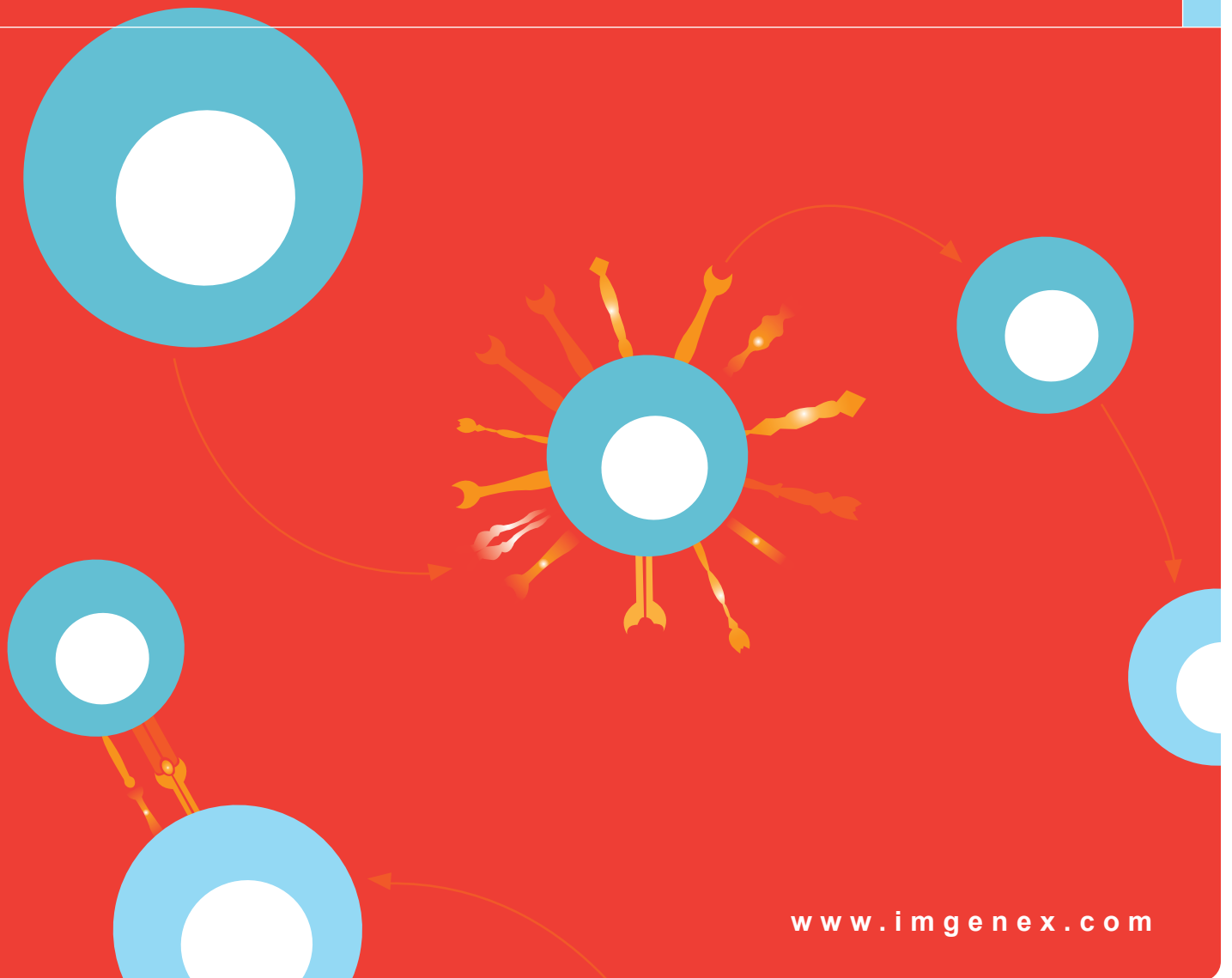


Regulatory T Cell

RESEARCH TOOLS



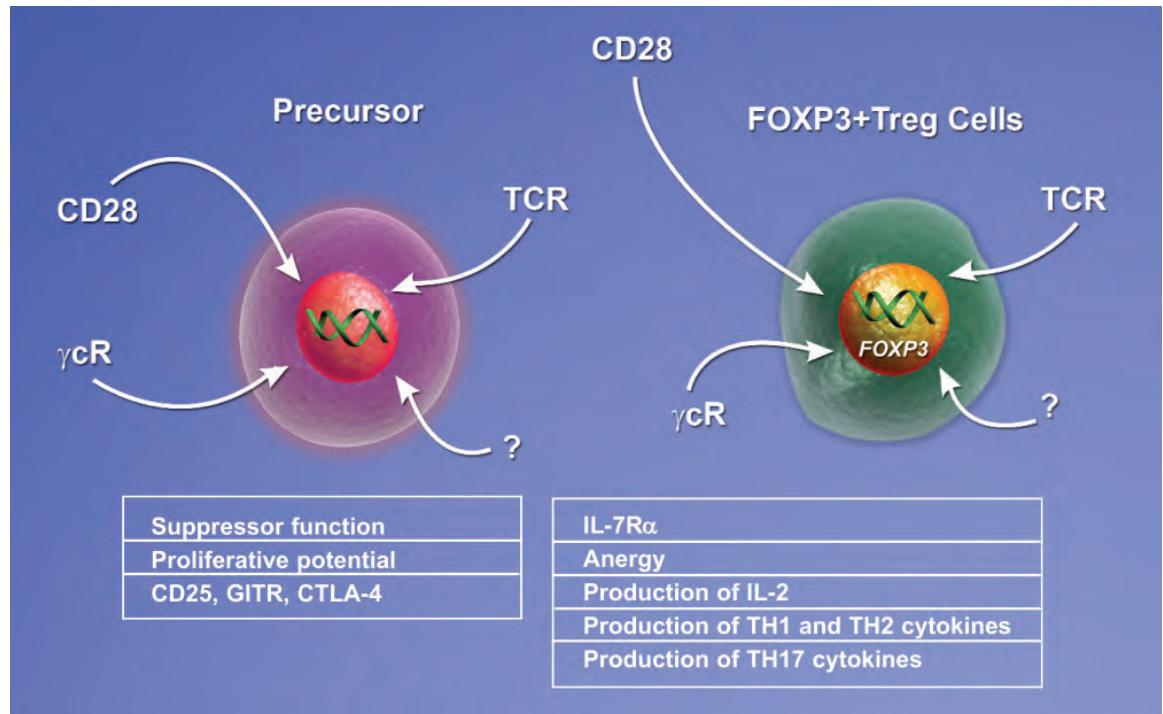
Regulatory T Cell

Early development and differentiation of nascent T cells, which migrate from bone marrow to become mature, naïve T cells, and are capable of responding to antigen, takes place inside the thymus. Around 10¹⁰ TCR (T cell receptor) variations are generated in developing T lymphocyte clones through a random process of somatic cell gene reorganization. During this process, T cells recognizing self-antigens can be generated. Due to the ability of these self-reactive T cells to elicit an autoimmune attack, they are permanently removed by the thymus through negative selection and clonal deletion. However, some of them manage to escape the thymic defenses and harbor themselves in the peripheral lymphoid organs. These T lymphocytes undergo further differentiation into effectors of various immune functions, or remain self-reactive, leading to various autoimmune diseases.

One of many immunotolerance mechanisms that the immune system has developed to distinguish between self and nonself antigens is regulatory T cells or Tregs. These cells are recently characterized specialized T cell subsets that actively suppress a variety of immune responses. Researchers have broadly classified Tregs into natural and adaptive Tregs. Natural Tregs are CD4+CD25+ T cells that originate in the thymus and play a significant role in immune homeostasis and protection against autoimmunity. Adaptive Tregs are generated in the periphery and may be induced by certain cytokines to become inducible Tregs during pathological and inflammatory conditions such as cancers and infections.

Although the principal immunosuppressive mechanism of Tregs remains elusive, several in vivo experimental models have indicated that Tregs secrete large amounts of immunosuppressants including IL-9, IL-10 and TGF- α upon activation.

Although the principal immunosuppressive mechanism of Tregs remains elusive, several in vivo experimental models have indicated that Tregs secrete large amounts of immunosuppressants including IL-9, IL-10 and TGF- α upon activation. These lymphokines are capable of inhibiting activation of Th1, Th2 cells and CTLs required for cell-mediated immunity, inflammation and antibody production. Certain recent experimental data and results even indicate that IL-2/IL-2R signaling is vital for development, maintenance, survival, expansion and suppressive activity of Tregs. Increased expression of certain other charac-



FOXP3 is induced in thymic precursor cells upon engagement with high-affinity TCR and other costimulatory factors resulting in FOXP3+ Treg cells. Different functions associated with Treg cell differentiation and function are shown in the boxes.

teristic markers including CTLA-4, glucocorticoid-inducible tumor necrosis factor receptor (GITR) and OX40 has been identified on Tregs whose function inside these cells is still not clear. The TCRs displayed on Tregs are capable of recognizing and interacting with any peptide-MHC class II ligand within a certain range of avidity but the contribution of TCR signaling and role of TCR-ligand interactions towards regulatory T cell development remains to be determined.

Several elegant experiments using transgenic mice, and retrovirus-mediated over expression studies, have led to the identification of FOXP3, a transcription factor, as a specific molecular marker essential for the development and function of Tregs. The primary evidence regarding the involvement of FOXP3 in the development of Tregs was provided in patients suffering from IPEX, a rare and fatal human autoimmune disorder. In these patients, a mutated FOXP3 gene causes improper development of Tregs resulting in hyperactivation of T cells reactive to self-antigens. Recently, experiments have clearly shown that retroviral

mediated introduction of FOXP3 into conventional CD4+ T cells converts them into regulatory T cells.

The emergence of regulatory T cells and the role of FOXP3 as a critical player in the negative control of various normal and pathological immune responses holds great promise for the development of novel therapies useful for the treatment of autoimmune diseases in humans. However, there are several questions that remain to be answered including the basic biology of the Tregs, various ligands responsible for thymic selection of these cells, the exact function of FOXP3 in relation with various markers present on Tregs and most importantly, the mechanisms by which Tregs exert their suppressive effects. A better understanding of manipulating FOXP3 and Tregs would enable us to harness the tremendous therapeutic potential in various clinical situations including Type I diabetes, Multiple sclerosis, GVHD, rheumatoid arthritis, allergy, and cancers.

TLR Expression in Tregs

A Treg is a CD4+ T cell that reduces or suppresses the immune responses of B cells or of other T cells to an antigen. Tregs were discovered in 1970 and coined “Suppressor T cells,” later the name was changed to “Regulatory T cell (Treg).” However, the understanding of Treg function remained nebulous following their initial discovery, and their suppressive ability was recognized as more of a phenomenon than a regulated process. About thirty years after their discovery, the concept of Tregs as integral components of the immune system began to take hold and a research area focused on Tregs emerged. Tregs are now key players in the global research effort to understand immune regulation (reviewed in van Maren et al, 2008 and Walker, 2009).

The current dogma puts forth that Tregs are vital for maintaining tolerance and homeostasis, and for preventing the immune system from becoming overactive through a highly tuned process. Additionally, Treg dysregulation can cause aberrant immune responses, and the absence of Tregs results in immune chaos.

Tregs can be broadly divided into two groups:

1. Tregs that originate in the thymus: referred to as ‘naturally occurring Tregs’
2. Tregs that develop in the periphery: referred to as ‘adaptive Tregs’

The most well characterized Tregs are the naturally occurring CD4+CD25+ Tregs, which constitute 5-15% of the total CD4+ T cell population. Naturally occurring Tregs express the transcription factor FOXP3 which is induced by TGF- β . FOXP3 appears to be the most specific marker for naturally occurring Tregs, although several other markers have been identified, including CTLA-4, GITR, CCR8 and the absence of CD127. Figure 26 is an example from Bell et al (2007) of using FOXP3 as a Treg marker to help characterize TLR expression in Tregs versus non-Tregs.

At least two types of adaptive CD4+ Tregs have been characterized, Type 1 regulatory (Tr1) and Type 3 regulatory (Th3) T cells. Tr1 cells arise after repeated TCR stimulation in the presence of IL-10. Th3 cells differentiate from naïve CD4+ T cell precursors through repeated TCR stimulation in combination with exposure to high levels of TGF- β . The field of Tregs is rapidly expanding, and it is likely additional Treg subsets remain to be discovered.

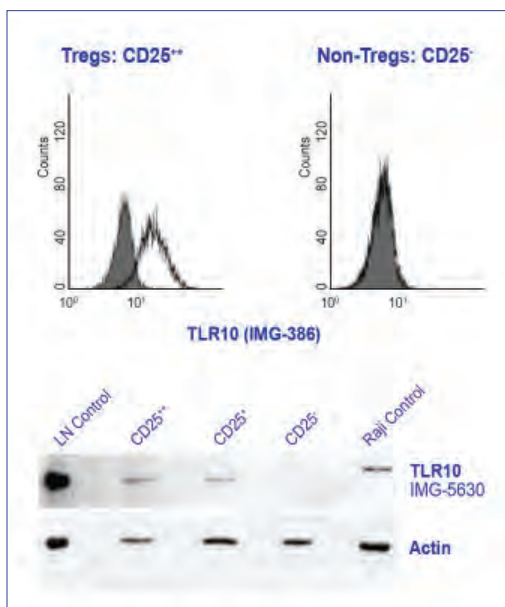


Figure 26. Comparison of TLR10 expression in human Treg and non-Treg cells by flow cytometry [(cell surface), IMG-386 (Clone 158C114)] and western blot analysis (IMG-5630).

Human Treg⁺ cells were isolated from healthy donors by Ficoll separation and magnetic bead sorting using a CD4+CD25+ Treg cell isolation kit. Cells of the highest CD25 expression (CD25++) were selected through incubation with a limiting quantity of anti-CD25 Ab beads. Non-Treg CD25⁻ cells were selected by collecting flow through from saturating amount of anti-CD25 Ab beads. Cells of intermediate CD25 expression (CD25+) were collected using a non-limiting (moderate) amount of CD25 Ab beads.

The data shows that TLR10 was detected in the Treg (CD4+CD25++ and CD4+CD25+) cell, but not the non-Treg (CD4+CD25-) cells. Normal lymph node (LN) and Raji cells were used as a positive western blot control for TLR10 expression. The Western Blot Loading Control Kit (IMG-6166K) containing a Beta Actin polyclonal antibody (IMG-5142A-050) is recommended for detecting actin or other housekeeping proteins.

* Treg CD4+CD25++ cells were >60% FOXP3 positive and non-Treg CD4+CD25- cells were <3% positive for FOXP3 by intracellular staining. The suppressive phenotype of the Treg CD4+CD25++ cells was confirmed through *in vitro* suppression assays. Source: Bell et al, 2007.

Both naturally occurring and adaptive Tregs monitor the activity of effector T cells (Teffs), where they regulate the initiation, expansion, and retraction of Teff responses. Teff responses must be closely controlled to prevent extensive immune-mediated tissue damage or autoimmune disease. Once activated, Tregs can suppress Teff proliferation and cytokine production as well as APC function, thereby controlling Teff responses. Tregs have been best characterized for regulating the activity of CD4+ Teffs; however they also influence the activity of CD8+ Teffs, B cells, and cells of the innate immune system.

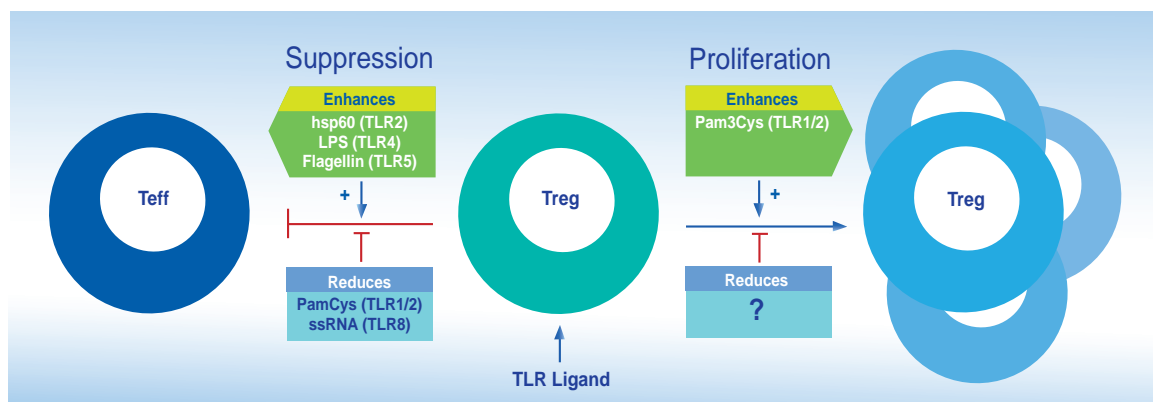


Figure 27. TLRs and modulation of Tregs. Ligand activated TLR signaling is thought to directly modulate Treg function. This includes enhancing or reducing Treg suppressive and/or proliferative ability. Adapted from Van Maren et al (2008).

The discovery that TLR signaling directly or indirectly regulates the suppressive capability of Tregs is among the farthest-reaching developments in the Treg field (reviewed in van Maren, 2009). The expression of TLRs in Tregs was first shown in 2003 by Caramaldo and her colleagues who demonstrated that a subset of CD4+ T cells known to exert regulatory functions expressed TLRs 4, 5, 7 and 8. Since then, TLR expression in Tregs has been reported in a number of studies. For example, Bell et al (2007) found that TLR10 was constitutively expressed in primary human Treg cells isolated from the peripheral blood of healthy donors as is shown in Figure 26.

Overall, TLR expression profiling studies have indicated that multiple TLRs are expressed in naturally occurring CD4+CD25+ Tregs. Additionally, TLR expression has been shown to be functionally relevant in various Treg model systems. Collectively, results suggest that as a family TLRs have both positive and negative effects on the suppressive function of CD4+CD25+ Tregs. That is, TLR signaling has been shown to both enhance and reduce the ability of these Tregs to suppress Teffs. Additionally, TLR signaling can also enhance Treg proliferation and may also act to inhibit it. These results are summarized below and reviewed in van Maren et al (2008):

- 1. Enhance Treg suppression**
 - a. Hsp60 (TLR2)
 - b. LPS (TLR4)
 - c. Flagellin (TLR5)
- 2. Reduce Treg suppression**
 - a. Pam3Cys (TLR1/2)
 - b. ssRNA (TLR8)

3. Enhance Treg proliferation

- Pam3Cys (TLR1/2)

4. Reduce Treg proliferation

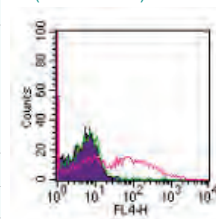
- Remains to be defined

A conceptual overview of ligands and TLRs with respect to modulation of Treg activity is shown in Figure 27. However, the role of TLR activation itself on Tregs as well as the mechanisms underlying modulation of Treg function, e.g. to suppress or not to suppress, remain to be fully elucidated. Critical questions regarding the dynamics of TLR expression on Treg subsets during homeostasis, infection and inflammation will need to be answered in order to understand the role of TLR expression on Tregs in both health and disease.

Elucidating relationships between TLRs, Tregs and cancer is of particular interest in the TLR/Treg field. Tregs can infiltrate tumors and suppress the anti-tumor response in mice, and increased levels of Tregs have been found in the peripheral blood of cancer patients. Both naturally occurring and adaptive Tregs have been found in tumor microenvironments. Since TLRs can reduce the suppressive activity of Tregs as shown in Fig 27, manipulating TLR signaling could potentially lift Treg suppression of the anti-tumor immune response. Hence, leveraging TLR signaling on Tregs may offer new opportunities for shifting the balance between tolerance and immunity, and lead to new treatments for cancer and other diseases.

Description	Cat No	Format	Clone	Host	Species	Application
CD3	IMG-5923A	Purified	145-2C11	Hamster	M	Flow-CS
	IMG-5923C	FITC	145-2C11	Hamster	M	Flow-CS
	IMG-5923D	PE	145-2C11	Hamster	M	Flow-CS
	IMG-5923E	Azide-Free	145-2C11	Hamster	M	FA-A, FA-D, Flow-CS
	IMG-5923G	APC	145-2C11	Hamster	M	Flow-CS
CD3	IMG-80315	T.C. Sup	F7.2.38	Mouse	H	IHC-P
CD3	IMG-80337	T.C. Sup	SP7	Rabbit	Multi	IHC-P, WB
CD3 e	IMG-80449	Purified	N/A	Rabbit	Multi	IHC-P
CD3 zeta	IMG-80450	Purified	N/A	Rabbit	H, M, R	IHC-P
CD3, T Cell	IMG-80081	T.C. Sup	PS1	Mouse	H	IHC-P
CD3, T Cell	IMG-80354	Purified	N/A	Rabbit	H	IHC-P
CD4	IMG-5808A	Purified	NA	Rabbit	Chimp, H	WB
CD4	IMG-5922A	Purified	GK1.5	Rat	M	Flow-CS
	IMG-5922C	FITC	GK1.5	Rat	M	Flow-CS
	IMG-5922D	PE	GK1.5	Rat	M	Flow-CS
	NEW IMG-5922E	Azide Free	GK1.5	Rat	M	Flow-CS
	IMG-5922G	APC	GK1.5	Rat	M	Flow-CS
CD4	IMG-80083	T.C. Sup	1F6	Mouse	H	IHC-P, WB
CD4 (L3T4)	IMG-5916A	Purified	RPA-T4	Mouse	H	FA-Neut, Flow-CS, IHC
	IMG-5916C	FITC	RPA-T4	Mouse	H	Flow-CS
	IMG-5916D	PE	RPA-T4	Mouse	H	Flow-CS
	IMG-5916E	Azide Free	RPA-T4	Mouse	H	FA-Neut, Flow-CS, IHC
	IMG-5916G	APC	RPA-T4	Mouse	H	Flow-CS
CD8	IMG-5917A	Purified	RPA-T8	Mouse	H	Flow-CS
	IMG-5917C	FITC	RPA-T8	Mouse	H	Flow-CS
	IMG-5917D	PE	RPA-T8	Mouse	H	Flow-CS
	IMG-5917G	APC	RPA-T8	Mouse	H	Flow-CS
CD8, T Cell	IMG-80331	T.C. Sup	144B	Mouse	H	IHC-P
CD8, T Cell	IMG-80344	T.C. Sup	SP16	Rabbit	H	IHC-P
CD8, T Cell	IMG-80436	Purified	N/A	Rabbit	H	IHC-P
CD25	IMG-5918A	Purified	7G7B6	Mouse	H	Flow-CS
	IMG-5918C	FITC	7G7B6	Mouse	H	Flow-CS
	NEW IMG-5918D	PE	7G7B6	Mouse	H	Flow-CS
	IMG-5918G	APC	7G7B6	Mouse	H	Flow-CS
	IMG-5918H	PerCP-Cy5.5	7G7B6	Mouse	H	Flow-CS
CD25	IMG-5924A	Azide-Free	PC61	Rat	M	Flow-CS
	IMG-5924C	FITC	PC61	Rat	M	Flow-CS
	IMG-5925D	PE	PC61	Rat	M	Flow-CS
	IMG-5924G	APC	PC61	Rat	M	Flow-CS
CD25, IL2 Receptor	IMG-80105	T.C. Sup	ACT-1	Mouse	H	Flow-CS, IHC-Fr
CD25, IL2 Receptor	IMG-80169	Purified	IL2R.1	Mouse	H	IHC-Fr, IHC-P
CD38	IMG-80205	T.C. Sup	AT13/5	Mouse	H	IHC-P
CD45, LCA	IMG-80026	T.C. Sup	2B11&PD7/26	Mouse	H	IHC-Fr, IHC-P

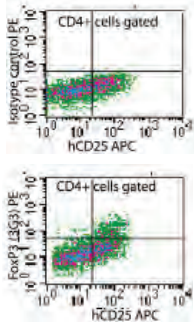
CD25
(IMG-5918G)



Flow analysis of CD25 in 10⁶ human PBMCs with 38 hour ConA stimulation (5 ug/ml) using 10 ul of IMG-5918G.

Description	Cat No	Format	Clone	Host	Species	Application
CD45RA, B Cell	IMG-80027	T.C. Sup	4KB5	Mouse	H	IHC-Fr, IHC-P
CD45RB	IMG-80172	T.C. Sup	Bra-11	Mouse	H	IHC-P
CD45RB	IMG-80173	T.C. Sup	DF-B1	Mouse	H	IHC-P
CD45RB, LCA	IMG-80028	T.C. Sup	PD7/26	Mouse	H	IHC-P
CD45RO, T Cell	IMG-80029	Purified	UCHL-1	Mouse	H	Flow-CS, IHC-Fr, IHC-P, IP, WB
CD54/ICAM-1	IMG-80108	Purified	15.2	Mouse	H	Flow, IHC-Fr, IHC-P, WB
CD62E/ELAM-1	IMG-80111	Purified	1.2B6	Mouse	H, Pig	Flow-CS, IHC-Fr, IHC-P, IP, WB
CD127/IL-7R	DDX0700*	Purified	R34-34	Mouse	H	FA-Neut, Flow-CS, IP
	DDX0700A488*	Alexa Fluor® 488	R34-34	Mouse	H	Flow-CS
	DDX0700A546*	Alexa Fluor® 546	R34-34	Mouse	H	IHC-P
	DDX0700A647*	Alexa Fluor® 647	R34-34	Mouse	H	Flow-CS, IHC-P
EBI3	NEW IMG-6100A	Purified	N/A	Rabbit	Chimp, H	WB
EBI3	NEW IMG-6185A	Purified	N/A	Rabbit	H, M	WB
EBI3	NEW IMG-6327A	Purified	N/A	Rabbit	M, R	IHC-P, WB
EBI3	NEW IMG-6706A	Purified	IMG10J706	Mouse	M	Flow-Intra, WB
EBI3	NEW IMG-6707A	Purified	IMG10J763	Mouse	M	Flow-Intra, WB
EBI3	NEW IMG-6708A	Purified	IMG10J803	Mouse	M	Flow-Intra, WB
EBI3	NEW IMG-6709A	Purified	IMG10J811	Mouse	M	Flow-Intra, WB
Fas (CD95)	IMG-240	Purified	DX2	Mouse	H	Flow
Fas (CD95)	IMG-80403	Purified	N/A	Rabbit	H	IHC-P
Folate Receptor 4	IMG-6217A	Purified	TH6	Rat	M	Flow-CS
	IMG-6217C	FITC	TH6	Rat	M	Flow-CS
	IMG-6217D	PE	TH6	Rat	M	Flow-CS
	IMG-6217E	Azide Free	TH6	Rat	M	Flow-CS
	IMG-6217G	APC	TH6	Rat	M	Flow-CS
Folate Receptor 4	IMG-6218A	Purified	12A5	Rat	M	Flow-CS
	IMG-6218C	FITC	12A5	Rat	M	Flow-CS
	IMG-6218D	PE	12A5	Rat	M	Flow-CS
	IMG-6218E	Azide Free	12A5	Rat	M	Flow-CS
	NEW IMG-6218G	APC	12A5	Rat	M	Flow-CS
FOXP3	IMG-5802A	Purified	3G3	Mouse	H, M	Flow-Intra, WB
	IMG-5802C	FITC	3G3	Mouse	H, M	Flow-Intra
	IMG-5802D	PE	3G3	Mouse	H, M	Flow-Intra
	IMG-5802G	APC	3G3	Mouse	H, M	Flow-Intra
FOXP3 Δ 2	IMG-6018A	Purified	10D4G6	Mouse	H	WB
	IMG-6018E	Azide Free	10D4G6	Mouse	H	WB
GITR	IMG-5920A	Purified	DTA-1	Rat	M	Flow-CS
	IMG-5920C	FITC	DTA-1	Rat	M	Flow-CS
	IMG-5920D	PE	DTA-1	Rat	M	Flow-CS
	IMG-5920G	APC	DTA-1	Rat	M	Flow-CS
GITRL	IMG-5474	P, AP	N/A	Rabbit	H, M	WB
GPR18	IMG-71505	P, AP	N/A	Rabbit	H	IHC-P
GPR83	IMG-5864A	P, AP	NA	Rabbit	Multi	IHC-P, IHC-Fr, WB
GPR83	IMG-5913A	P, AP	N/A	Rabbit	Multi	IHC-P, IHC-Fr, WB
GPR83	IMG-71559	P, AP	N/A	Rabbit	H	IHC-P
GPR83	IMG-71560	P, AP	N/A	Rabbit	H	IHC-P
GPR83	IMG-71561	P, AP	N/A	Rabbit	H, M	Flow-CS, IHC-P
GPR83	IMG-71562	P, AP	N/A	Rabbit	H	IHC-P
GRAIL/RNF128	IMG-6058A	P, AP	N/A	Rabbit	Multi	WB

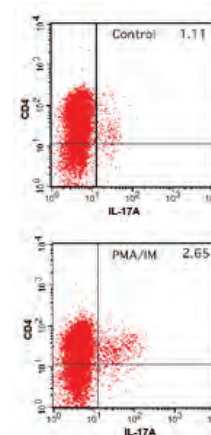
**FOXP3/Scurfin
(IMG-5802D)**



Flow cytometric analysis of FOXP3 in human PBMCs stimulated with anti-hCD3 and rhIL-2 (IMR-248) for 48 hours using isotype control (top) and IMG-5802D (bottom) at 0.1 ug/10⁶ cells.

Description	Cat No	Format	Clone	Host	Species	Application
IL-10	IMG-222B	Biotin	JES5-16E3	Rat	M	ELISA, Flow-Intra, WB
IL-10	IMG-223A	Purified	JES5-2A5	Rat	M	ELISA, ELISPOT, WB
	IMG-223B	Biotin	JES5-2A5	Rat	M	ELISA, ELISPOT, WB
IL-10	IMG-317A	Purified	JES3-9D7	Rat	H	ELISA, FA-Neut, Flow-Intra, WB
	IMG-317E	Azide Free	JES3-9D7	Rat	H	ELISA, FA-Neut, Flow-Intra, WB
IL-10	IMG-318A	Purified	JES3-12G8	Rat	H	ELISA, IHC-Fr
IL-17A	IMG-6545A	Purified	4K5F6	Mouse	H	Flow-Intra, WB
	IMG-6545A647	Alexa Fluor® 647	4K5F6	Mouse	H	Flow-Intra
	IMG-6545B	Biotin	4K5F6	Mouse	H	Flow-Intra, WB, ELISA
	IMG-6545D	PE	4K5F6	Mouse	H	Flow-Intra
IL-17A	NEW IMG-6580A	Purified	4H1524	Mouse	H	Flow-Intr, WB
	NEW IMG-6580AF488	Purified	4H1524	Mouse	H	Flow-Intr, WB
IL-17B/E R	IMG-345A	Purified	97C691	Mouse	H	WB
IL-17B/E R	IMG-470A	Purified	N/A	Rabbit	H	WB
IL-17E	IMG-323A	Purified	68C1039.2	Mouse	H, M	WB
IL-17F	NEW IMG-6578A	Purified	4H450	Mouse	H, M	WB
IL-17F	NEW IMG-6579A	Purified	4H1629	Mouse	H, M	IHC-P, WB
IL-33/IL-1F11	IMG-6016A	P, AP	N/A	Rabbit	Chimp, H	WB
Integrin, Alpha X	IMG-72062	P, AP	N/A	Rabbit	H	IHC-P
Integrin, Alpha X	IMG-72063	P, AP	N/A	Rabbit	H	IHC-P
KLF4/GKLF	IMG-3231	P, AP	N/A	Rabbit	H	IHC-Fr, WB
KLF4/GKLF	NEW IMG-6081A	Purified	N/A	Rabbit	H	Flow-Intra, IHC-P WB
Neuropilin-1	DDX0440*	Purified	211H6.01	Mouse	H	Flow-CS, IHC-Fr
	DDX0440A488*	Alexa Fluor® 488	211H6.01	Mouse	H	Flow-CS, IHC-Fr
	NEW DDX0440A647*	Alexa Fluor® 647	211H6.01	Mouse	H	Flow-CS, IHC-Fr
NFATc1	NEW IMG-4073	Purified	N/A	Rabbit	H	WB
NFATc1	NEW IMG-4074	Purified	N/A	Rabbit	H	WB
NFATc1	NEW IMG-5101A	Purified	N/A	Rabbit	H	WB
NFATc2	IMG-4075	Purified	N/A	Rabbit	H	WB
PTPRC	IMG-72065	P, AP	N/A	Rabbit	H	IHC-P
Qa-1b	IMG-6223A	Purified	6A8.6F10.1A6	Mouse	M	Flow-CS
ROR Gamma	IMG-6013A	P, AP	N/A	Rabbit	Chimp, H	IHC-P
ROR γ /ROR γ (t)	NEW IMG-6275A	Purified	4G419	Rabbit	Chimp, H, M	Flow-Intra, WB
	NEW IMG-6275A488	Alexa Fluor® 488	4G419	Rabbit	Chimp, H, M	Flow-Intra, WB
	NEW IMG-6275C	FITC	4G419	Rabbit	Chimp, H, M	Flow-Intra, WB
	NEW IMG-6275D	PE	4G419	Rabbit	Chimp, H, M	Flow-Intra, WB
	NEW IMG-6275G	APC	4G419	Rabbit	Chimp, H, M	Flow-Intra, WB
ROR Gamma	IMG-71896	P, AP	N/A	Rabbit	H	IHC-P
TGFb3	IMG-80481	Purified	N/A	Rabbit	M, R	IHC-P
TIM-3/HAVCR2	IMG-5284A	Purified	N/A	Rabbit	M	WB
TIM-3	NEW IMG-6543A	Purified	F38-2E2	Mouse	H	Flow-CS
	NEW IMG-6543D	PE	F38-2E2	Mouse	H	Flow-CS
	NEW IMG-6543E	Azide-Free	F38-2E2	Mouse	H	Flow-CS
TIM-3	NEW IMG-6544D	PE	B8.2C12	Mouse	M	Flow-CS
TLR2/CD282	IMG-319	Ascites	1030A5.138	Mouse	H	Flow-CS+Intra, IHC-P, WB
TLR2/CD282	IMG-410A	Purified	N/A	Rabbit	H, M	IHC-P, WB
	IMG-410E	Azide-Free	N/A	Rabbit	H, M	WB

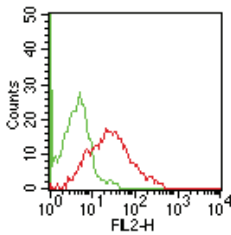
IL-17A
(IMG-6580AF488)



Intracellular flow cytometric analysis of IL-17A in human PBMCs (lymphocytes) stimulated with PMA (5 ng/ml), Ionomycin (1 mM), and Brefeldin A (1X) for 5 hrs, using CD3+ cells and analyzed with IMG-6580AF488-100 (red) at 10 ul/10⁶ cells (green represents isotype control). IMGENEX's intracellular flow kit (10083K) was used for this test.

Description	Cat No	Format	Clone	Host	Species	Application
TLR2/CD282	IMG-416A	Purified	TL2.1	Mouse	Dog, H	Flow-CS+Intra, IF/ICC, IHC-P, IP, WB
	IMG-416B	Biotin	TL2.1	Mouse	Dog, H	Flow-CS+Intra, IF/ICC, IP
	IMG-416AF488	Alexa Fluor® 488	TL2.1	Mouse	Dog, H	Flow-CS+Intra, IF/ICC
	IMG-416AF647	Alexa Fluor® 647	TL2.1	Mouse	Dog, H	Flow-CS+Intra, IF/ICC
	IMG-416C	FITC	TL2.1	Mouse	Dog, H	Flow-CS+Intra, IF/ICC
	IMG-416D	PE	TL2.1	Mouse	Dog, H	Flow-CS+Intra, IF/ICC
	IMG-416E	Azide-Free	TL2.1	Mouse	Dog, H	FA-Neut, Flow-CS+Intra, IF/ICC, IP
TLR2/CD282	IMG-526	Purified	N/A	Rabbit	H, M	WB
TLR2/CD282	IMG-545	Purified		Rabbit	Chimp, H, M, R	WB
TLR2/CD282	IMG-5651	Purified	N/A	Rabbit	H, M	IHC-P, WB
TLR2/CD282	IMG-6320A	Purified	T2.5	Mouse	H, M	FA-Neut, Flow-CS, IHC-Fr, IP
	IMG-6320C	FITC	T2.5	Mouse	H, M	FA-Neut, Flow-CS, IHC-Fr, IP
TLR2/CD282	IMG-662	Purified	N/A	Rabbit	M	WB
TLR2/CD282	IMG-545E	Azide-Free	N/A	Rabbit	Multi-species	WB
TLR4/CD284	IMG-6285A	PAP	N/A	Rabbit	Multi-species	WB
TLR4/CD284	IMG-6307A	PAP	N/A	Rabbit	Multi-species	WB
TLR4/CD284	IMG-417A	Purified	HTA125	Mouse	Dog, H	CM, FA-Neut, Flow-CS+Intra, IF/ICC, IP
	IMG-417AF647	Alexa Fluor® 647	HTA125	Mouse	Dog, H	CM, Flow-CS, Flow-Intra
	IMG-417B	Biotin	HTA125	Mouse	Dog, H	CM, ELISA, FA-Neut, Flow-CS+Intra, IF/ICC, IP
	IMG-417C	FITC	HTA125	Mouse	Dog, H	Flow-CS+Intra
	IMG-417D	PE	HTA125	Mouse	Dog, H	CM, Flow-CS+Intra
	IMG-417E	Azide-Free	HTA125	Mouse	Dog, H	CM, FA-Neut, Flow-CS+Intra, IF/ICC, IP
TLR4/CD284	IMG-428A	Purified	MTS510	Rat	M	Flow-CS, IP
	IMG-428AF647	Alexa Fluor® 647	MTS510	Rat	M	Flow-CS
	IMG-428C	FITC	MTS510	Rat	M	Flow-CS
	IMG-428D	PE	MTS510	Rat	M	Flow-CS
	IMG-428E	Azide-Free	MTS510	Rat	M	FA-Neut, Flow-CS, IP
TLR4/CD284	IMG-5031A	Purified	76B357.1	Mouse	H, M, R	Flow-CS+Intra, IHC-P, WB
	IMG-5031AF647	Alexa Fluor® 647	76B357.1	Mouse	H, M, R	Flow-CS+Intra
	IMG-5031C	FITC	76B357.1	Mouse	H, M, R	Flow-CS+Intra
	IMG-5031D	PE	76B357.1	Mouse	H, M, R	Flow-CS+Intra
TLR4/CD284	IMG-577	Purified	N/A	Rabbit	H, M	IP, WB
TLR4/CD284	IMG-578A	Purified	N/A	Rabbit	H, M	WB
TLR4/CD284	IMG-579A	Purified	N/A	Rabbit	H, M	Flow-CS, IHC-Fr, IHC-P, WB
TLR4/CD284 (Tyr674)	IMG-6276A	PAP	N/A	Rabbit	H, M, R	WB
TLR5	IMG-580	Purified	N/A	Rabbit	H, M, R	Flow-Intra, IHC-P, WB
TLR5	IMG-663A	Purified	85B152.5	Mouse	Dog, H, M	Flow-CS+Intra, WB
	IMG-663C	FITC	85B152.5	Mouse	Dog, H, M	Flow-CS+Intra
	IMG-663D	PE	85B152.5	Mouse	Dog, H, M	Flow-CS+Intra

TIM-3
(IMG-6543D)



Cell surface staining of Con A stimulated human PBMCs with 10 ul of PE-conjugated human TIM-3 antibody (Red) and mouse IgG1 isotype control (Green) (IMGENEX, 20106). IMGENEX's cell surface flow kit (10084K) was used for this test (cells were not fixed for testing).

Description	Cat No	Format	Clone	Host	Species	Application
TLR5	IMG-664A	Purified	19D759.2	Mouse	Dog, H, M	Flow-CS+Intra, IHC-Fr, IHC-P, WB
	IMG-664AF488	Alexa Fluor® 488	19D759.2	Mouse	Dog, H, M	Flow-CS+Intra
	IMG-664AF647	Alexa Fluor® 647	19D759.2	Mouse	Dog, H, M	Flow-CS+Intra
	IMG-664C	FITC	19D759.2	Mouse	Dog, H, M	Flow-CS+Intra
	IMG-664D	PE	19D759.2	Mouse	Dog, H, M	Flow-CS+Intra
TLR5	IMX-5131	Sera	N/A	Rabbit	H	ELISA
TLR7	DDX0500	Purified	66H3	Mouse	H, M, R	Flow-Intra
TLR7	IMG-540	Purified	N/A	Rabbit	H, M	WB
TLR7	IMG-5632	Purified	N/A	Rabbit	H, M	WB
TLR7	IMG-581A	Purified	N/A	Rabbit	H, M	Flow-CS, IF/ICC, IHC-Fr, IHC-P, IP, WB
	IMG-581C	FITC	N/A	Rabbit	H, M	Flow-CS+Intra
	IMG-581E	Azide-Free	N/A	Rabbit	H, M	Flow-CS, IF/ICC, IHC-Fr, IHC-P, IP, WB
TLR7	IMG-6070A	Purified	N/A	Rabbit	Multi-species	Flow-Intra, WB
TLR7	IMG-665A	Purified	N/A	Rabbit	H, M	Flow-CS+Intra, IHC-Fr, WB
	IMG-665C	FITC	N/A	Rabbit	H, M	Flow-CS+Intra
	IMG-665D	PE	N/A	Rabbit	H, M	Flow-CS+Intra
TLR7	NEW IMG-6675A	Purified	IMG4G6	Mouse	H	Flow-Intra, WB
	NEW IMG-6675AF488	Alexa Fluor® 488	IMG4G6	Mouse	H	Flow-Intra, WB
	NEW IMG-6675D	PE	IMG4G6	Mouse	H	Flow-Intra, WB
TLR8/CD288	DDX0480*	Purified	303F1.14	Mouse	H	Flow-Intra, IF/ICC, IHC-Fr, WB
	DDX0480A488*	Alexa Fluor® 488	303F1.14	Mouse	H	Flow-Intra, IF/ICC
	DDX0480A546*	Alexa Fluor® 546	303F1.14	Mouse	H	IF/ICC
	DDX0480B*	Biotin	303F1.14	Mouse	H	Flow-Intra, IF/ICC, IHC-Fr, WB
TLR8/CD288	DDX0483*	Purified	112H7.15	Rat	H	Flow-Intra, IHC (Bouin), WB
	DDX0483A488*	Alexa Fluor® 488	112H7.15	Rat	H	Flow-Intra, IF/ICC
	DDX0483A546*	Alexa Fluor® 546	112H7.15	Rat	H	IF/ICC
	DDX0483B*	Biotin	112H7.15	Rat	H	Flow-Intra, IF/ICC, WB
TLR8/CD288	IMG-321A	Purified	44C143	Mouse	H, M	Flow-CS+Intra, IHC-P, WB
	IMG-321AF488	Alexa Fluor® 488	44C143	Mouse	H, M	Flow-CS+Intra
	IMG-321AF647	Alexa Fluor® 647	44C143	Mouse	H, M	Flow-CS+Intra
	IMG-321B	Biotin	44C143	Mouse	H, M	ELISA
	IMG-321C	FITC	44C143	Mouse	H, M	Flow-CS+Intra
	IMG-321D	PE	44C143	Mouse	H, M	Flow-CS+Intra
TLR8/CD288	IMG-5653-1	Purified	N/A	Rabbit	H, M	IF/ICC, WB
TLR10/CD290	IMG-386A	Purified	158C1114	Mouse	H	Flow-CS+Intra, WB
	IMG-386C	FITC	158C1114	Mouse	H	Flow-CS+Intra
	IMG-386D	PE	158C1114	Mouse	H	Flow-CS+Intra
TLR10/CD290	IMG-5255A	Purified	N/A	Rabbit	M, R	ELISA, WB
TLR10/CD290 (IN)	IMG-5630	Purified	N/A	Rabbit	H	WB

FOXP3 Staining Kits

Description	Cat No	Quantity
FOXP3 Flow Intracellular Staining Buffer Set	10086K	1 kit
Human FOXP3 (APC Conjugate) Staining Assay Kit	10092K	25 Tests
Human FOXP3 (PE Conjugate) Staining Assay Kit	10091K	25 Tests
Mouse FOXP3 (APC Conjugate) Staining Assay Kit	10095K	25 Tests
Mouse FOXP3 (PE Conjugate) Staining Assay Kit	10094K	25 Tests

RNA Interference

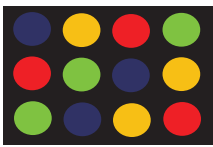
Description	Cat No	Quantity
FOXP3 (human) siRNA ReadyGene (target sequence 125-1350 nt) in the pSuppressorRetro plasmid vector; suitable for generating human FOXP3 knockdown permanent cell lines. Target sequence nts. 1250-1300 of human FOXP3.	IMG-1003	1 kit
FOXP3 (human) siRNA ReadyGene in the pSuppressor plasmid vector; suitable for generating human FOXP3 knockdown permanent cell lines. Target sequence nts. 1100-1200 of human FOXP3.	IMG-826	1 kit
FOXP3 (human) siRNA ReadyGene in the pSuppressor plasmid vector; suitable for generating human FOXP3 knockdown permanent cell lines. Target sequence nts. 1250-1350 of human FOXP3.	IMG-827	1 kit
FOXP3 (human) siRNA ReadyGene in the pSuppressorRetro plasmid vector; suitable for generating human FOXP3 knockdown permanent cell lines. Target sequence nts. 1100-1200 of human FOXP3.	IMG-1002	1 kit

Lysate and Expression System

Description	Cat No	Quantity
FOXP3 Cell Lysates (transfected & mock transfected)	40209	2 vials
FOXP3, human (full length cDNA) in PCLXSN Retroviral Expression Vector	10049P	2 µg

References

- Control of regulatory T cell development by the transcription factor FOXP3. Hori Nomura T, Sakaguchi S. *Science* 299 (5609):1057-1061 (2003).
- IPEX and the role of FOXP3 in the development and function of human Tregs. Le Bras and Geha RS. *J Clin Invest*. 116(6):1473-1475 (2006).
- Genome-wide analysis of FOXP3 target genes in developing and mature regulatory T cells. Zheng Y, Josefowicz SZ, Kas A, Chu TT, Gavin MA and Rudensky AY. *Nature* 445 (7130): 936-940 (2007).
- FOXP3 in control of the regulatory T cell lineage. Zheng Y, Rudensky AY. *Nat Immunol*. 8(5): 457-462 (2007).
- Human CD4+ T Cells Express TLR5 and Its Ligand Flagellin Enhances the Suppressive Capacity and Expression of FOXP3 in CD4+CD25+ T Regulatory Cells Crellin NK, Garcia RV, Hadisfar O, Allan SE, Steiner TS, and Levings MK. *J Immunol*, 175: 8051-8059 (2005).
- Forkhead Box P3 Regulates TLR10 Expression in Human T Regulatory Cells. Bell MP, Svingen PA, Rahman MK, Xiong Y, and Faubion WA Jr. *J Immunol*, 179, 1893 -1900 (2007).



Screening Services for Inflammation & Immune Signaling Pathways

IMGENEX is a recognized leader in the provision of tools for studying cellular responses. We have experience in the manufacturing of reagents and development of applications and assay systems for studying pathways of cellular response, particularly pathways related to Immune Receptors with a focus on Toll-like Receptors. Over the past several years IMGENEX has developed a broad product portfolio in the area of Toll-like Receptors (TLRs), and related areas of Inflammation and Immunology providing advanced research tools to accelerate the increasing volume of applied research in these areas of biology.

In parallel with their critical role in shaping and regulating cellular and immune responses, is the recognition of Toll-like Receptors as tunable targets to control these responses. Identifying new, more specific and more potent TLR agonists or antagonists is an area of

focus for companies with intent for commercial development of diagnostics for monitoring response, or interested in therapeutics as prophylactic or curative drugs.

To help meet these objectives we now offer our new IMGENEX Screening Services as a partner with expertise in cell signaling areas, as evidenced by the portfolio of products already commercialized. We are recognized as authorities in cell signaling pathways especially in the area of NF-κB related signaling pathways which are directly related to Inflammation and Immune Signaling Pathways.

Through IMGENEX Screening Services our objective is to become your partner in accelerating your research and product development efforts and meeting your specific objectives efficiently and economically in accord with the scope, resources, and timetable structure desired.

Partnering Information & Initiation of IMGENEX Screening Services Project

1 Contact IMGENEX Screening Services

Tel: 858-642-0978 x 244
Toll-Free: 888-723-4363 x 224
info@imgenex.com

2 Assurance of Confidentiality

Exchange NDA assures confidentiality

3 Discussion of Overall Project

Project Scope, Timetable, Objectives

4 IMGENEX provides SOW-Statement of Work

Systems, Assays, Reagents Planned

Data Analysis Formats and Requirements

Timetable & Milestones
Quotation Presentation

5 In Depth Discussion and Project Review

Review and revise SOW as agreed upon

6 Agreement and Go Ahead

Start partnering with IMGENEX

Toll-like Receptor	Key Ligands	Anti-Microbial			Adapter Molecules			Cell Location			Signaling Pathway		
		MyD88	TRAM	TRIF	TRAM	TRAM	TRAM	IRF3	IRF3	IRF7	IRF3	IRF7	IRF3
TLR1	Lipids, Diacyl Lipopeptides, Peptidoglycan	✓					✓						
TLR2	Lipids, Peptidoglycan	✓					✓						
TLR3	dsRNA, Poly(I):Poly(C)						✓						
TLR4	LPS	✓					✓						
TLR5	Flagellin	✓					✓						
TLR6	Lipids, Diacyl Lipopeptides, Peptidoglycan	✓					✓						
TLR7	ssRNA						✓						
TLR8	ssRNA						✓						
TLR9	CpG-DNA						✓						
TLR10	Oryzanol						✓						
TLR11	Uropathogenic Bacteria	✓					✓						
TLR12	?						✓						
TLR13	?						✓						

TLR & Inflammatory Ligand Discovery Screen

- Identification of TLR ligands in cell culture
- Identification of TLR ligands in tissue fluids
- Identification of TLR specific ligands

NF-κB SEAPorter™ Assay Kit (Cat. No. IMK-515)

The IMGENEX NF-κB SEAPorter™ Assay Kit is designed to provide a flexible read-out system for the measurement of NF-κB activation, by ELISA compatible techniques, via quantitative measurement of secreted alkaline phosphatase in cell supernatants.

The NF-κB SEAPorter™ Assay Kit offers

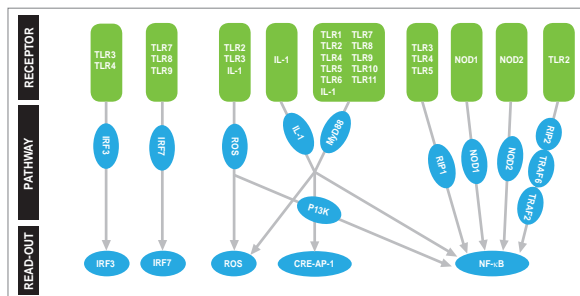
- High-throughput screening
- Convenient 96 well format
- Choice of cells
- Start to finish in as little as 1 hour
- Complete with reagents including plasmids containing SEAP cDNA, buffers, and substrate.

NF-κB Transfected HEK 293 Stable Cells (Cat. No. IML-101)

IMGENEX now provides cells which are stably transfected with an NF-κB inducible secreted alkaline phosphatase gene designed as an **optimal** choice of cells for our new SEAPorter™ Assay Kit.

TLR & Inflammatory Ligand Activation Screen

- Pathway Analysis
- Activation Potential



TLR & Inflammatory Pathway Analysis

- Mediator Cascade Analysis
- Cytokines
- Chemokines
- Prostaglandins
- Interferons

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