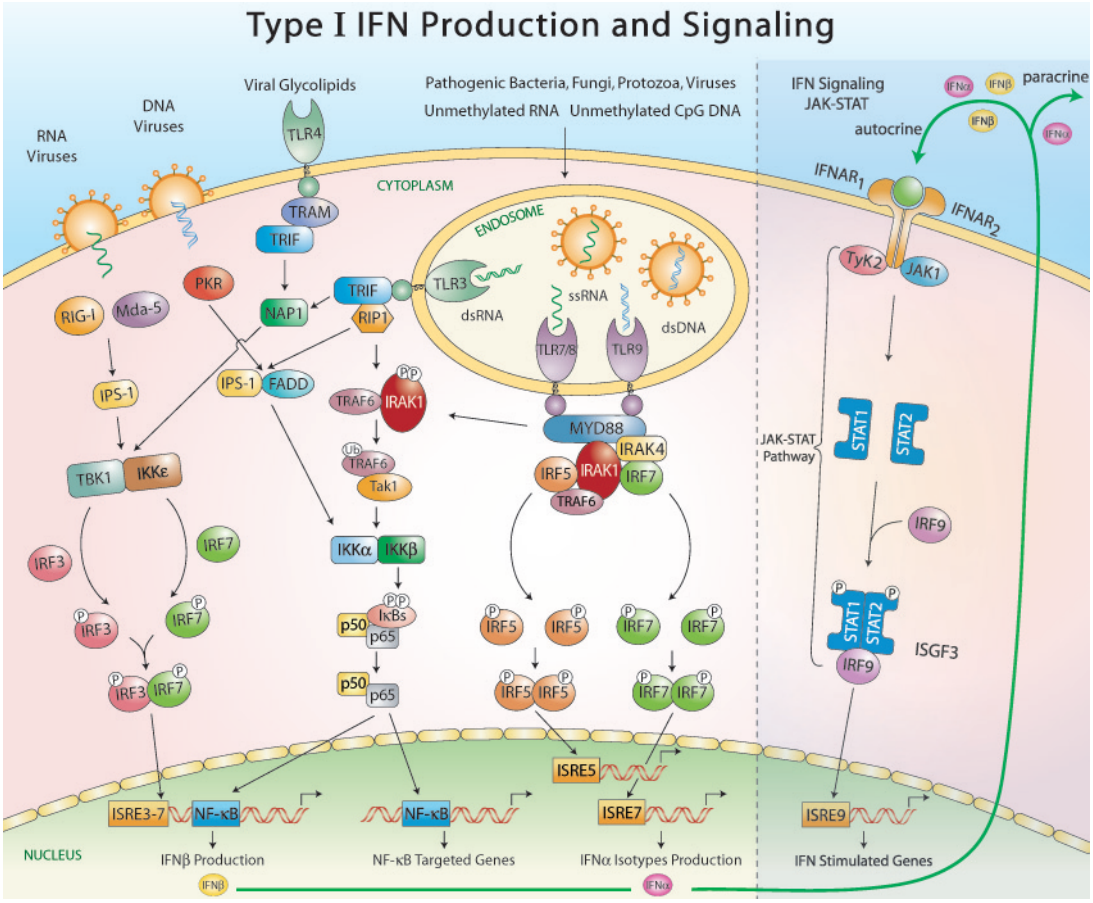
September/October 2005

InvivoGen Insight

InvivoGen provides a complete set of tools to study Type I IFN pathways. All the genes of the cascades are available in pUNO plasmids, some of them are HA-tagged to facilitate their study. In addition, most of the dominant negative and dominant positive forms of these genes described in the literature are offered. Numerous validated plasmid-based siRNAs are also available. InvivoGen provides recombinant IFNα/β and an extensive list of TLR ligands that can be used to stimulate Type I IFN pathways. Finally, a choice of promoters activated by IFN regulatory factors (IRFs) can be studied by determining SEAP production upon induction. The newly released QUANTI-Blue™ solution used to measure SEAP activity completes this range of products.

Inside this issue:



The most studied members of the Type I family of interferons are the multiple IFN α isotypes and IFN ß. Type I IFNs are responsible for inducing transcription of a large group of genes which play a role in host resistance to viral infections, as well as activating key components of the innate and adaptive immune systems including antigen presentation and production of cytokines involved in activation of T cells, B cells, and natural killer cells.

Type I IFNs are transcriptionally regulated, and are induced following recognition of pathogen components during infection by various host pattern recognition receptors. Virtually all humans cells are able to synthesize IFNα/ß, however some cells have a more pronounced ability to produce these cytokines. Table 1 summarizes some characteristics of the three main pathways leading to the production of Type I IFN. The RIG-I pathway is activated upon infection by RNA viruses. The second pathway involves the adaptor protein TRIF which is recruited by TLR3 and TLR4. The last pathway is triggered by TLR7/8 and TLR9 leading to the activation of the transcription factor IRF7. Following their production, Type I IFNs trigger antiviral responses by binding to a common receptor (IFNAR). IFNα/ß binding to IFNAR stimulates the JAK1-STAT pathway leading to the assembly of the ISGF3 complex which is composed of STAT1-STAT2 dimers and IRF9. ISGF3 binds to IFN-stimulated response elements (ISRE) in the promoters of IFN-stimulated genes to regulate their expression. Among these genes is IRF7 which initiates the transcription of a second wave of Type I IFNs. This autocrine/paracrine feed-back allows Type I IFNs to create an antiviral state in surrounding cells.

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The Pathways of Type I IFN Production

PATHWAYS	MAIN ACTORS	LOCALIZATION	INDUCERS	CELLS	REFERENCES
RIG-I pathway	RIG-I (MDA-5)	Cytoplasmic	Many single- and double-stranded RNA viruses	Conventional DCs Fibroblasts, Hepatocytes	1, 2, 3, 4, 5, 6
TRIF pathway	TLR3-TRIF TLR4-TRIF	Internal vesicles Plasma membrane	Unmethylated dsRNA Viral glycolipids	Macrophages, Hepatocytes	4, 5, 6, 7, 8
IRF7 pathway	TLR7/8-MyD88-IRF7(IRF5) TLR9-MyD88-IRF7(IRF5)	Endosome	Unmethylated RNA from pathogens and damaged host cells Unmethylated CpG DNA Chromatin immuno complexes	Plasmacytoid DCs	8, 9, 10

Table 1: Three major pathways involved in IFNα/ß production in humans and mice.

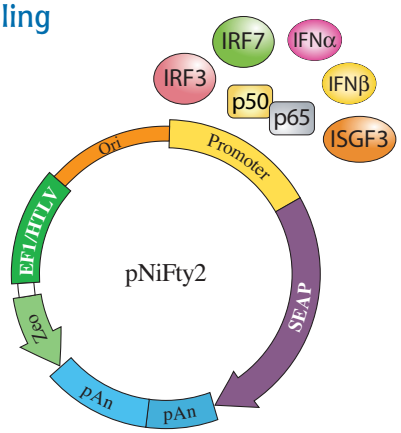
References:

1. Kato H. *et al*., 2005. Cell type-specific involvement of RIG-I in antiviral response. Immunity. 23(1):19-28.
2. Yoneyama M. *et al*., 2005. Shared and Unique Functions of the DExD/H-Box Helicases RIG-I, MDA5, and LGP2 in Antiviral Innate Immunity. J Immunol. 175(5):2851-8.
3. Kawai T. et *al*., 2005. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated Type I interferon induction. Nat Immunol. [Epub ahead of print]
4. Li K. *et al*., 2005. Distinct poly(I-C) and virus-activated signaling pathways leading to interferon-beta production in hepatocytes. J Biol Chem. 280(17):16739-47.
5. Perry AK. *et al*., 2005. The host Type I interferon response to viral and bacterial infections. Cell Res 15:407.
6. Gale M. Jr, Foy EM., 2005. Evasion of intracellular host defence by hepatitis C virus. Nature. 436(7053):939-45.
7. Schroder M., Bowie AG., 2005. TLR3 in antiviral immunity: key player or bystander? Trends Immunol. 26(9):462-8.
8. Kariko K. *et al*., 2005. Suppression of RNA Recognition by Toll-like Receptors: The Impact of Nucleoside Modification and the Evolutionary Origin of RNA. Immunity. 23(2):165-75.
9. Honda K. *et al*., 2005. IRF-7 is the master regulator of Type-I interferon-dependent immune responses. Nature. 434(7034):772-7.
10. Rifkin IR. *et al*., 2005. Toll-like receptors, endogenous ligands, and systemic autoimmune disease. Immunol Rev. 204:27-42. Review.

Inducible Promoters

Highly Inducible Promoters for the study of Type I IFN Production and Signaling

Interferons are key modulators of the immune response. Their pleiotropic activities are mediated by the induction of many IFN-stimulated genes (ISGs). To help study the transcriptional regulation and signal transduction of Type I IFNs, InvivoGen provides several reporter systems, called pNiFty2, based on the inducible expression of the secreted embryonic alkaline phosphatase (SEAP) gene. The SEAP gene is cloned under the control of three different promoters that are activated by various transcription factors, such as IRF3, IRF5, IRF7 and NF-κB.



pNiFty2-IFA-SEAP features the **mouse IFNα4** minimal promoter[1]. Transcription of mIFNα4 is mediated by a virus responsive element (VRE-A4) located in the promoter. VRE-A4 contains four cooperating DNA modules that bind to IRF3 and IRF7[2]. Transfection of HEK293 cells with pNiFty2-IFA-SEAP followed by stimulation with the Newcastle Disease virus (NDV) resulted in high SEAP induction.

pNiFty2-IFB-SEAP features the **mouse IFNß** minimal promoter, which comprises several positive regulatory domains (PRDs) that bind different cooperating transcription factors such as NF-κB, IRF3 and IRF7[3]. Co-expression of IFNß-SEAP with TLR3, TLR7/8 or TLR9 and induction with the corresponding ligand in HEK293 cells led to a strong increase in SEAP expression.

pNiFty2-56K-SEAP features the **human ISG-56K** minimal promoter. ISG-56K is the prototype ISG which expression is highly increased by Type I IFNs due to the presence of ISRE and IRF7 sites within the promoter[4]. pNiFty2-56K-SEAP provides a good model to study the JAK-STAT pathway. HEK293 cells transfected with pNiFty2-56K-SEAP and stimulated with IFNα/ß required the cotransfection of both pUNO-STAT2 and pUNO-IRF9, as they appear deficient for this pathway, to produce high levels of SEAP.

pNiFty2 plasmids are selectable by Zeocin™ in both *E. coli* and mammalian cells. They are provided as transformed *E. coli* strains on paper disk with 4 pouches of Fast-Media™ Zeo.

1. Braganca J. *et al*., 1997. Synergism between multiple virus-induced factor-binding elements involved in the differential expression of interferon A genes. J Biol Chem. 272(35):22154-62.
2. Morin P. *et al*., 2002. Preferential binding sites for interferon regulatory factors 3 and 7 involved in interferon-A gene transcription. J Mol Biol. 316(5):1009-22.
3. Vodjdani G. *et al*., 1988. Structure and characterization of a murine chromosomal fragment containing the interferon beta gene. J Mol Biol. 204(2):221-31.
4. Der SD. *et al*., 1998. Identification of genes differentially regulated by interferon alpha, beta, or gamma using oligonucleotide arrays. PNAS. 95(26):15623-8.

Product	Code
pNiFty2-IFA-SEAP	pnf2-ifasp
pNiFty2-IFB-SEAP	pnf2-ifbsp
pNiFty2-56K-SEAP	pnf2-56ksp

TLR Signaling and IRF Activation Pathways

InvivoGen offers an extensive set of tools to study the TLR signaling and IRF activation pathways. In particular, InvivoGen provides a comprehensive choice of TLR ligands, recombinant Types I and II interferons and all the genes involved in these pathways. These genes are available native, tagged to facilitate their detection, or mutated to act either as dominant positives or negatives. Furthermore, InvivoGen has designed, constructed and tested plasmid-based siRNAs (shRNAs) to silence some of the key genes of these pathways.

Genes and shRNAs

Gene Name	Native Genes	HA-tagged Genes	Dominant Positives	Dominant Negatives	shRNAs
TLR1 to 13	✓	✓		✓	✓
IRF1	✓	✓			✓
IRF3	✓	✓	✓		✓
IRF5	✓	✓	✓		✓
IRF7	✓	✓	✓		✓
IRF9	✓	✓			✓
IRAK-1	✓	✓		✓	✓
IRAK-4	✓				✓
MyD88	✓	✓		✓	✓
TIRAP	✓			✓	✓
TRAF6	✓	✓		✓	✓
TRAM	✓			✓	✓
TRIF	✓		✓	✓	✓
FADD	✓			✓	
IKKα	✓	✓			
IKKβ	✓				
IKKε	✓				
MDA-5	✓				
NEMO	✓	✓			
PKR	✓			✓	✓
RIG-I	✓			✓	✓
RIP1	✓				
TBK1	✓				
IFNAR1	✓				✓
IFNAR2	✓				
JAK1	✓				
STAT1	✓				✓
STAT2	✓				✓
TYK2	✓				

Native Genes

Intronless genes cloned from the ATG to the Stop codon, provided in pUNO, a blasticidin-selectable plasmid.

HA-tagged Genes

Genes fused at the 3'end to 3 motifs of the influenza hemaglutinine (HA) tag, provided in pUNO, a blasticidin-selectable plasmid.

Dominant Positives

Mutant genes constitutively activated, provided in pUNO, a blasticidin-selectable plasmid.

Dominant Negatives

Mutant gene that blocks the activity of the normal, wild-type gene, provided in pDeNy, a Zeocin™-selectable plasmid, or pZERO (TLR genes) a puromycin-selectable plasmid.

shRNAs

Short hairpin RNAs targeting genes involved in the activation of IRFs, provided in psiRNA, a Zeocin™-selectable plasmid.

Check our website for gene descriptions, catalog codes, and updated lists of native and genes modified as well as plasmid-based siRNAs

Interferons

InvivoGen provides human Type I and Type II interferons produced by recombinant DNA technology. They are fully proficient in inducing the JAK-STAT pathway in susceptible cell lines.

Source: cDNA obtained from human mRNAs, expressed in *E. coli* or CHO (IFNß1a)
Purity: Greater than 98%
Formulation: Lyophilized
Activity: Recombinant IFNs are fully biologically active compared to standards.

Product	Species	Quantity	Code
IFNα2a	human	20 µg	hifn-a2a
IFNα2b	human	20 µg	hifn-a2b
IFNß1a	human	2 µg	hifn-b1a
IFNß1b	human	2 µg	hifn-b1b
IFNγ	human	20 µg	hifn-g

More information on interferons and TLRs ligands available on our website.

QUANTI-Blue™

Determination & Quantification of Secreted Alkaline Phosphatase

QUANTI-Blue™ is a colorimetric enzyme assay developed to determine secreted alkaline phosphatase (SEAP) activity in supernatants of cell cultures. The SEAP gene is a truncated form of human placental alkaline phosphatase a GPI-anchored protein and is widely used to study promoter activity or gene expression. QUANTI-Blue™ offers many advantages over the conventional SEAP Detection Kit (#rep-sap) based on the pNPP substrate:

Requires small samples of cell supernatants

Samples of 10 μl are sufficient.

No need to process samples

Preparation of cell lysates or heating of samples is not required.

Determine SEAP activity without disturbing cells

The same cell cultures can be repeatedly sampled for kinetic studies or further experimentation.

Assay can be completed in 30 min

Hands-on time no longer than 10 min. The enzymatic activity can be detected as early as 20 min after incubation of the samples in QUANTI-Blue™.

Wide dynamic range allows to detect low and high levels of SEAP

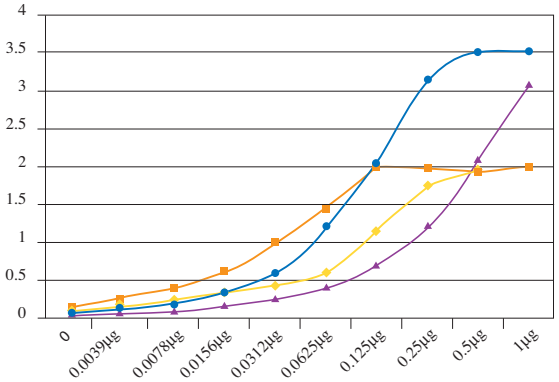
No need to perform multiple sample dilutions.

Extremely simple to use

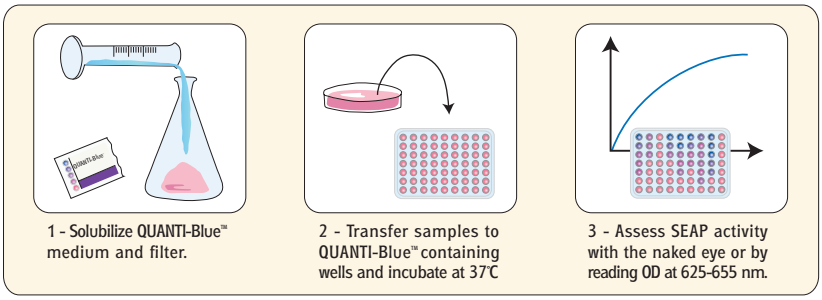
QUANTI-Blue™ consists of only one medium: 1) resuspend in water, 2) add sample, incubate at 37°C and 3) read OD.

Highly sensitive for quantitative measurement

Higher saturation threshold than with pNPP resulting in more significant differences between non or low SEAP expression and high SEAP expression.



Comparison QUANTI-Blue™ and pNPP: Purified SEAP was serially diluted in QUANTI-Blue™ or assessed using the SEAP Detection Kit (pNPP), and incubated at 37°C for 30 minutes or 2 hours. To determine SEAP activity, OD was read at 405 nm (pNPP) or 655 nm (QUANTI-Blue™). Legend: ◆pNPP 30 min, ■pNPP 2 hours, ▲QUANTI-Blue™ 30 min, ●QUANTI-Blue™ 2 hours.



1 - Solubilize QUANTI-Blue™ medium and filter.

2 - Transfer samples to QUANTI-Blue™ containing wells and incubate at 37°C

3 - Assess SEAP activity with the naked eye or by reading OD at 625-655 nm.

Product	Quantity	Code
QUANTI-Blue™	5 pouches	rep-qb1
	10 pouches	rep-qb2

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