InvivoGen Insight
INNOVATION WITHIN REACH
FALL 2010

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REVIEW

Recognition of cytosolic DNA

Recognition of Cytosolic DNA

The innate immune system reacts to diverse molecules of microbial origin, termed pathogen-associated molecular patterns (PAMPs), or released from damaged or dying cells, called damage-associated molecular patterns (DAMPs). These molecules include nucleic acids, such as DNA. While the recognition of extra-cellular DNA involves mainly Toll-like receptor 9, recognition of cytosolic DNA appears to involve several sensors.

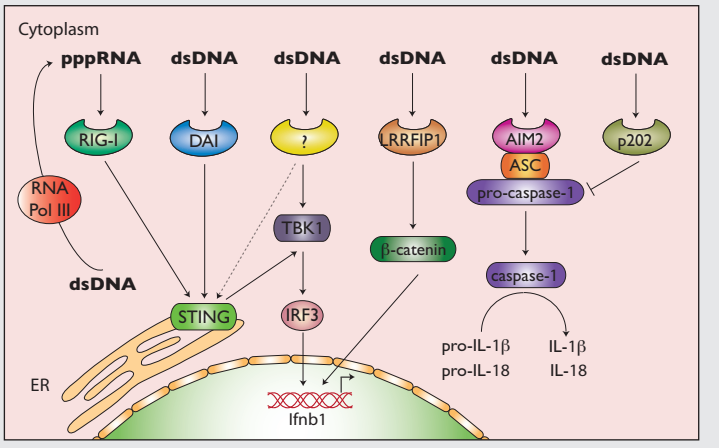
The first identified cytosolic DNA sensor, named DNA-dependent activator of IFN-regulatory factors (DAI), binds cytosolic dsDNA and leads to the production of type I IFNs[1]. However, DAI defficiency does not affect the induction of type I IFNs in response to poly(dA:dT), a synthetic analog of B-DNA, suggesting that redundant cytosolic DNA sensors exist[2]. Unexpectedly, the next candidate receptor was the RNA helicase retinoic acid-inducible gene-I (RIG-I), an RNA-binding and not DNA-binding protein. A human cell line deficient for RIG-I was shown to lack the ability to recognize poly(dA:dT)[3]. Recently, two independent teams confirmed the involvement of RIG-I in the response to dsDNA and demonstrated that rather than the cytosolic DNA, an RNA intermediate was responsible for RIG-I activation. They found that transfected poly(dA:dT) is transcribed by RNA polymerase III in the cytoplasm and potentially in the nucleus into a double-stranded RNA intermediate. This dsRNA molecule contains a 5'-triphosphate moiety and is recognized by RIG-I[4, 5]. Both DAI and RIG-I induce the production of type I IFNs through the TBK1/IRF3 pathway. The endoplasmic reticulum (ER)-resident transmembrane protein stimulator of IFN genes (STING) is a key component of this pathway[6]. STING seems to function as an adaptor protein upstream of TBK1.

Recently, a third IFN-inducer cytosolic dsDNA sensor has been identified[7]. This sensor LRRFIP1 can recognize AT-rich B-form dsDNA as well as GC-rich Z-form dsDNA. With the use of LRRFIP1-specific siRNA, Yang *et al.* demonstrated that LRRFIP1 triggers the production of IFN-β in a β-catenin-dependent manner. β-Catenin binds to the C-terminal domain of IRF3 inducing an increase in IFN-β expression.

Although the production of type I IFNs is the main pathway induced by cytosolic dsDNA, production of the pro-inflammatory cytokines IL-1β and IL-18 has also been observed. Recently, several groups have identify AIM2 (absent in melanoma 2), a member of the hematopoietic interferon-inducible nuclear protein HIN-200 family, as a cytosolic dsDNA sensor which activation promotes the assembly of an inflammasome[8-10]. DNA of various origins, such as poly(dA:dT), plasmidic DNA and DNA from the bacterium *L. monocytogenes* have been shown to activate AIM2[11]. Upon activation, AIM2 interacts with ASC, a common adapter of the inflammasomes, leading to the cleavage of caspase-1 and the secretion of IL-1β and IL-18. p202 is another member of the HIN200 family shown to bind cytoplasmic dsDNA but, in contrast to AIM2, it represses caspase activation[12].

The recognition of cytosolic DNA is more complicated than first anticipated. Several sensors have been identified that trigger different signaling pathways in a cell type-specific manner. Still, the general consensus is that another unknown cytosolic DNA-recognition system, independent of the TLRs and RIG-I, may exist. Further studies to elucidate the complex mechanisms of cytosolic DNA recognition may help the development of new strategies to treat inflammatory diseases.

1. Takaoka A. *et al.*, 2007. DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. Nature. 448(7152):501-5. **2. Ishii KJ. *et al.*, 2008.** TANK-binding kinase-1 delineates innate and adaptive immune responses to DNA vaccines. Nature. 451(7179):725-9. **3. Cheng G. *et al.*, 2007.** Double-stranded DNA and double-stranded RNA induce a common antiviral signaling pathway in human cells. Proc Natl Acad Sci U S A. ;104(21):9035-40. **4. Ablasser A. *et al.*, 2009.** RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. Nat Immunol. 10(10):1065-72. **5. Chiu YH. *et al.*, 2009.** RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. Cell. 138(3):576-91. **6. Ishikawa H. & Barber GN., 2008.** STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature. 455(7213):674-8. **7. Yang P. *et al.*, 2010.** The cytosolic nucleic acid sensor LRRFIP1 mediates the production of type I interferon via a beta-catenin-dependent pathway. Nat Immunol. 11(6):487-94. **8. Hornung V. *et al.*, 2009.** AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature. 458(7237):514-8. **9. Fernandes-Alnemri T. *et al.*, 2009.** AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. Nature. 458(7237):509-13. **10. Bürckstümmer T. *et al.*, 2009.** An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. Nat Immunol. 10(3):266-72. **11. Jones JW. *et al.*, 2010.** Absent in melanoma 2 is required for innate immune recognition of Francisella tularensis. PNAS, 107(21):9771-6. **12. Roberts TL. *et al.*, 2009.** HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. Science ;323(5917):1057-60.



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Cytosolic dsDNA Sensors (CDS)

InvivoGen provides an extensive set of tools to study the sensors of cytosolic double-stranded (ds)DNA. These tools include synthetic analogs of dsDNA, a collection of human and mouse genes involved in the response to cytosolic dsDNA, CDS inhibitory molecules, and a reporter cell line.

Synthetic dsDNA Analogs - CDS Ligands

• Poly(dA:dT) (B-DNA)

Poly(dA:dT) is a repetitive synthetic double-stranded DNA sequence of poly(dA-dT)•poly(dT-dA) and a synthetic analog of B-DNA. Poly(dA:dT) is recognized by several sensors, including DAI, LRRFIP1 and AIM2. It has also been shown to be transcribed by RNA polymerase III into dsRNA with a 5'-triphosphate moiety (**5'ppp-dsRNA**) which is a ligand for RIG-I.

• Poly(dG:dC) (Z-DNA)

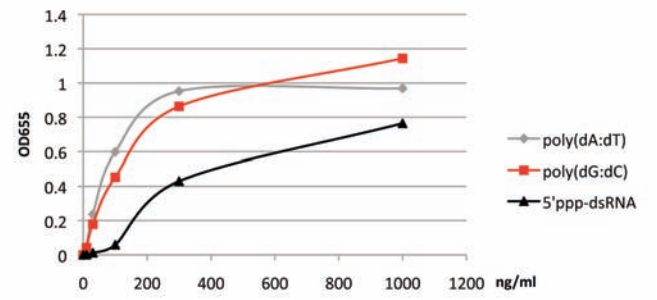
Poly(dG:dC) is a repetitive synthetic double-stranded DNA sequence of poly(dG-dC)•poly(dC-dG). Poly(dG:dC) is a synthetic analog of the Z-DNA form. It has been reported to be recognized by LRRFIP1.

Poly(dA:dT) and poly(dG:dC) are available naked or complexed with the cationic lipid LyoVec™ to facilitate their uptake. Their activity has been tested using the reporter cell line **B16-Blue™ IFNα/β** (see next page).

5'ppp-dsRNA is a short (19 mer) blunt-end double-stranded RNA with a 5' triphosphate. Transfected 5'ppp-dsRNA is a ligand of RIG-I. B16-Blue™ IFNα/β cells produce type I IFNs in response to transfected 5'ppp-dsRNA (see graph).

Contents and Storage

Poly(dA:dT) and poly(dG:dC), naked or complexed, and 5'ppp-dsRNA are provided lyophilized. Products are shipped at room temperature and should be stored at -20°C.



Responses of B16-Blue™ IFNα/β cells to dsDNA and dsRNA: B16-Blue™ IFNα/β cells were stimulated with increasing concentrations of poly(dA:dT), poly(dG:dC) or 5'ppp-dsRNA complexed extemporaneously with the transfection reagent LyoVec™. After 24h incubation, induction of type I IFNs was assessed by determining the levels of SEAP using QUANTI-Blue™, a SEAP detection medium.

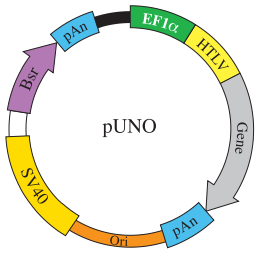
PRODUCT	QTY	CAT. CODE
Poly(dA:dT) naked NEW	200 µg 1 mg	tlrl-patn tlrl-patn-1
Poly(dA:dT) / LyoVec™	100 µg	tlrl-patc
Poly(dG:dC) naked NEW	200 µg	tlrl-pgcn
Poly(dG:dC) / LyoVec™ NEW	100 µg	tlrl-pgcc
5' ppp-dsRNA NEW	100 µg	tlrl-3prna-100

pUNO plasmids - CDS Genes

CDS and CDS-related genes are provided in a pUNO plasmid which contains the complete coding sequence from the ATG to the Stop codon (www.invivogen.com/orfs). Each gene is fully sequenced. pUNO plasmids are resistant to blasticidin.

Contents and Storage

Each pUNO plasmid is provided as a lyophilized transformed *E. coli* strain on a paper disk. Transformed strains are shipped at room temperature and should be stored at -20°C. Each pUNO plasmid is supplied with 4 pouches of *E. coli* Fast-Media® Blas.



Genes available:

- ASC
- DAI
- LRRFIP1
- STING
- AIM2
- IRF3
- RIG-I
- TBK1

Genes known to inhibit the pathways triggered by CDSs are also available.

PRODUCT	QTY	CAT. CODE*
pUNO-<gene>	*E. coli* disk	puno-<gene>

* Catalog codes are available on our website

BX795 and shRNAs - CDS Inhibitors

• BX795

BX795, an aminopyrimidine compound, was developed as an inhibitor of 3-phosphoinositide-dependent kinase 1 (PDK1). It was recently shown to be a potent inhibitor of the IKK-related kinases, TANK-binding kinase 1 (TBK1) and IKKε, and hence of IRF3 activation and IFN-β production. BX795 inhibits the catalytic activity of TBK1/IKKε by blocking their phosphorylation.

• shRNAs (Ready-made psiRNA plasmids)

shRNAs that target and silence by >70% CDS and CDS-related genes are expressed by ready-made psiRNA plasmids (www.invivogen.com/readymade-psirna). Their silencing efficiency is tested using the psiTEST system (www.invivogen.com/psitest-system).

Contents and Storage

BX795 is provided as a solid. Ready-made psiRNA plasmids are provided as 20 µg of lyophilized DNA. Products are shipped at room temperature. Store at -20°C.

PRODUCT	QTY	CAT. CODE
BX795	5 mg	tlrl-bx7
Ready-made psiRNA plasmid	20 µg	psirna42-<gene>

B16-Blue™ IFNα/β - IFNα/β Reporter Cell Line

Description

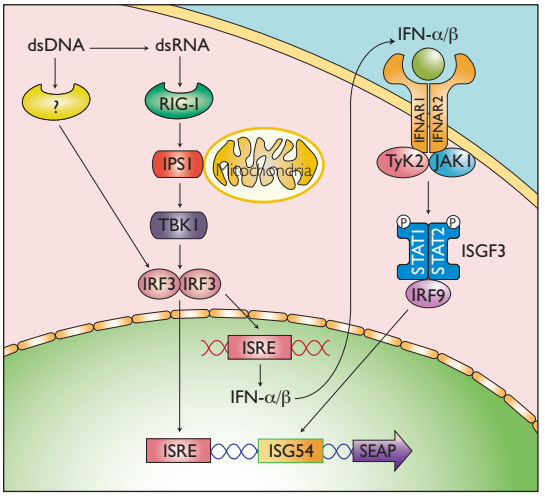
B16-Blue™ IFN-α/β cells allow the detection of bioactive murine type I IFNs by monitoring the activation of the JAK/STAT/ISGF3 and/or IRF3 pathway. B16-Blue™ IFN-α/β cells derive from the murine B16 melanoma cell line of C57B1/6 origin. It was stably transfected with a SEAP reporter gene under the control of the IFN-α/β-inducible ISG54 promoter enhanced by five Interferon Stimulated Response Elements (ISRE).

Stimulation of B16-Blue™ IFN-α/β cells with murine IFN-α or IFN-β, or type I IFN inducers, such as tranfected poly(dA:dT) or 5'ppp-dsRNA, activates the JAK/STAT/ISGF3 and IRF3 pathways leading to the production of SEAP. Levels of SEAP in the supernatant can be easily determined with QUANTI-Blue™, a medium that turns purple/blue in the presence of SEAP and by reading the OD at 655 nm.

Contents

B16-Blue™ IFN-α/β cells are grown in RPMI medium, 2 mM L-glutamine, 10% FBS supplemented with 100 µg/ml Zeocin™. Each vial contains 5-7x 10^6 cells and is supplied with 10 mg Zeocin™. Cells are shipped on dry ice.

InvivoGen also provides **HEK-Blue™ IL-1β cells** that provide a convenient read-out of IL-1β. In the presence of IL-1β a signaling cascade is activated inducing the production of SEAP.



JAK/STAT and IRF3 signaling pathways in B16-Blue™ IFNα/β

PRODUCT	QUANTITY	CAT. CODE
B16-Blue™ IFN-α/β cells	5-7 x 10^6 cells	bb-ifnab
HEK-Blue™ IL-1β cells	5-7 x 10^6 cells	hkb-il1b

Tagged Genes - pSELECT-Tag plasmids

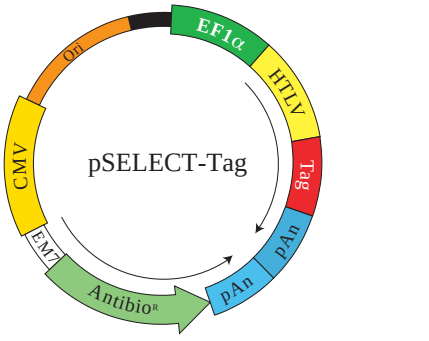
pSELECT-Tag is a new family of expression plasmids designed to generate tagged proteins in order to facilitate their detection and/or purification. pSELECT-Tag features three well-known tags: the green fluorescent protein (GFP) gene, the human influenza hemagglutinin (HA) epitope and the polyhistidine (His) tag. pSELECT-Tag plasmids can be used to generate either N-tagged or C-tagged proteins.

Description

pSELECT-Tag plasmids feature three commonly-used tags; GFP-Tag, HA-Tag and His-Tag. These tags can be added either at the N or C terminus of the protein of interest. The N-terminal tag encompasses the Start Codon and is followed by a multiple cloning site (MCS). The C-terminal tag is cloned downstream of an MCS and followed by a Stop codon.
pSELECT-Tag plasmids are available with the blasticidin or Zeocin™ selectable markers that confer antibiotic resistance in both *E. coli* and mammalian cells.

Applications

- GFP-Tag: Visualization of the spatial and temporal localization of the tagged protein by fluorescence microscopy
- HA-Tag: Detection of the tagged protein by immunocytochemistry, immunoprecipitation or Western blotting
  Purification of the tagged protein by affinity chromatography
- His-Tag: Purification of the tagged protein using an NI-NTA column



PRODUCT		QTY	CAT. CODE (N-Tag)	CAT. CODE (C-Tag)
GFP Tag	**pSELECT-GFP-blasti**	20 µg	psetb-ngfp	psetb-cgfp
	pSELECT-GFP-zeo	20 µg	psetz-ngfp	psetz-cgfp
HA Tag	**pSELECT-HA-blasti**	20 µg	psetb-nha	psetb-cha
	pSELECT-HA-zeo	20 µg	psetz-nha	psetz-cha
His Tag	**pSELECT-His-blasti**	20 µg	psetb-nhis	psetb-chis
	pSELECT-His-zeo	20 µg	psetz-nhis	psetz-chis

Contents

pSELECT-Tag plasmids are provided as 20 µg of lyophilized DNA and supplied with 4 pouches of *E. coli* Fast-Media® containing the appropriate selective antibiotic (2 TB and 2 Agar). Products are shipped at room temperature.

TLR and NOD Reporter Cells

InvivoGen introduces HEK-Blue™ TLR and HEK-Blue™ NOD cells, a collection of engineered cell lines designed to provide a rapid, sensitive and reliable method to screen and validate TLR and NOD agonists or antagonists. They express an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene that can be conveniently monitored using the SEAP detection media QUANTI-Blue™ or HEK-Blue™ Detection.

HEK-Blue™ TLR cells

HEK-Blue™ TLR cells are engineered HEK293 cells that stably co-express a human TLR gene and an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. To increase the sensitivity to their cognate agonists, HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells were further transfected with the co-receptors CD14 and MD2/CD14, respectively.
HEK-Blue™ TLR cells are resistant to the selective antibiotics blasticidin and Zeocin™. HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells are additionally resistant to hygromycin.

HEK-Blue™ NOD cells

HEK-Blue™ NOD cells are engineered HEK293 cells that stably co-express the human NOD1 or NOD2 gene and an NF-κB-inducible SEAP reporter gene.
HEK-Blue™ NOD cells are resistant to blasticidin and Zeocin™.

HEK-Blue™ Null cells

HEK-Blue™ Null cells are the parental cell lines used to generate HEK-Blue™ TLR and HEK-Blue™ NOD cells.

- **HEK-Blue™ Null1 cells** express the SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB binding sites. This cell line is the parental cell line of HEK-Blue™ TLR2, TLR3, TLR5, TLR8, TLR9 and NOD1 cells.
- **HEK-Blue™ Null1-k cells** express the same reporter system than HEK-Blue™ Null1 cells but are slightly different genotypically. This cell line is the parental cell line of HEK-Blue™ TLR7 cells.
- **HEK-Blue™ Null2 cells** express the SEAP reporter gene under the control of the IL-12 p40 minimal promoter fused to five NF-κB binding sites. This cell line is the parental cell line of HEK-Blue™ TLR4 and NOD2 cells.

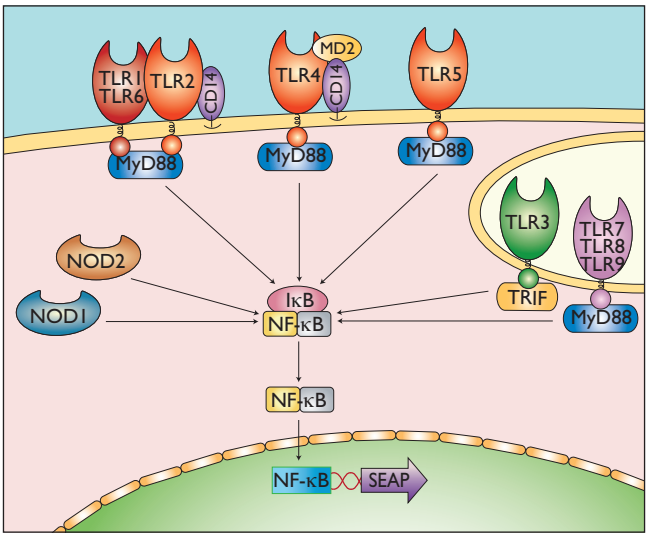
HEK-Blue™ Null cells are resistant to Zeocin™.

Principle

Recognition of a TLR or NOD agonist by its cognate receptor triggers a signaling cascade leading to the activation of NF-κB and the production of SEAP (figure). SEAP levels can be determined spectrophotometrically using HEK-Blue™ Detection or QUANTI-Blue™, both are SEAP detection media that turn purple/blue in the presence of alkaline phosphatase.

Contents

HEK-Blue™ TLR, HEK-Blue™ NOD and HEK-Blue™ Null cells are grown in DMEM medium, 2mM L-glutamine, 10% FBS and supplemented with 100 µg/ml Zeocin™, 30 µg/ml blasticidin and/or 200 µg/ml HygroGold™ (ultra-pure hygromycin) depending on the cell line. Cells are provided frozen in a cryotube containing 5-7 x 10^6 cells and supplied with the corresponding antibiotic(s) (10 mg Zeocin™, 1 mg blasticidin and/or 10 mg HygroGold™) and 1 pouch of QUANTI-Blue™. Cells are shipped on dry ice.



TLR- and NOD-induced NF-κB activation in HEK-Blue™ TLR and HEK-Blue™ NOD cells

Data for each HEK-Blue™ cell line is available on our website

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue™ TLR Cells		
HEK-Blue™ hTLR2 Cells	5-7 x 10^6 cells	hkb-htlr2
HEK-Blue™ hTLR3 Cells	5-7 x 10^6 cells	hkb-htlr3
HEK-Blue™ hTLR4 Cells	5-7 x 10^6 cells	hkb-htlr4
HEK-Blue™ hTLR5 Cells	5-7 x 10^6 cells	hkb-htlr5
HEK-Blue™ hTLR7 Cells	5-7 x 10^6 cells	hkb-htlr7
HEK-Blue™ hTLR8 Cells	5-7 x 10^6 cells	hkb-htlr8
HEK-Blue™ hTLR9 Cells	5-7 x 10^6 cells	hkb-htlr9
HEK-Blue™ NOD Cells		
HEK-Blue™ hNOD1 Cells	5-7 x 10^6 cells	hkb-hnod1
HEK-Blue™ hNOD2 Cells	5-7 x 10^6 cells	hkb-hnod2
HEK-Blue™ Null Cells		
HEK-Blue™ Null1 Cells	5-7 x 10^6 cells	hkb-null1
HEK-Blue™ Null1-k Cells	5-7 x 10^6 cells	hkb-null1k
HEK-Blue™ Null2 Cells	5-7 x 10^6 cells	hkb-null2

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