TLR7 & TLR8: fraternal twins

Toll-like receptors (TLRs) play a pivotal role in the initiation of anti-infectious immune responses. Distinct pathogen-associated molecular patterns (PAMPs) are recognized by different TLRs, at the cell surface or in endosomes. TLR7 and TLR8 are endosomal receptors that share structural homology and sense viral single stranded (ss) RNA as well as synthetic base analogs. However, there are functional differences between these two TLRs.

The endosomal distribution of TLR7 and TLR8 allows them to scan for the presence of microbial RNA in the phagocytic cargo. Their activation leads to NF-κB, AP1- and IRF-mediated production of type I IFNs (IFN-α/β) and pro-inflammatory cytokines. Structural analyses have revealed that both TLR7 and TLR8 possess two binding sites which do not share the same specificities. Site 1 is highly conserved between TLR7 and TLR8 and binds nucleosides (guanosine (G) for TLR7 and uridine (U) for TLR8) or base analogs. The ligand preference for TLR7 and TLR8 is explained by the presence of specific residues in Site 1. Site 2 is less conserved and binds ssRNA with U(U) and U(G) motifs, respectively. Of note, Site 1 occupancy allows the receptor dimerization, and signaling with ad hoc ligand concentration. ssRNA-binding to Site 2 is not sufficient for the formation of a signaling competent TLR dimer but it strongly enhances the binding affinity of Site 1. Thus, TLR7 and TLR8 appear to sense distinct RNA-degradation products rather than full-length ssRNAs.

TLR7 has been less studied than TLR8 as it was initially thought to be non-functional in mice. Of note, this does not hold true when using TLR8-506, an analog of the synthetic agonist VX-2337 (see inside). Further, TLR13 has been suggested as a murine TLR8 homolog. Thus, findings regarding mouse TLR7 and TLR8 are not transposable to their human counterparts. However, there is a renewed interest in TLR8 supported by the structural analyses, along with a report describing human TLR8 as a key sensor of bacterial viability through the recognition of bacterial RNA. TLR7 and TLR8 exhibit different expression patterns. TLR7 is essentially expressed by plasmacytoid dendritic cells (pDCs) but is also found in B cells and myeloid cells. TLR8 is absent from pDCs and B cells, and is highly expressed by myeloid cells. This suggests that TLR7 and TLR8 have evolved to mediate distinct immune responses upon microbial encounters. Viral infections trigger TLR7-mediated production of IFN-α in pDCs. However, in monocytes, TLR7 and TLR8 activation induces the expression of TNF-α and TLR1-7 and TLR1-8, respectively. Upon bacterial infection, TLR7 drives IFN-α production by pDCs, but its role in myeloid cells remains obscure. On the other hand, TLR8 seems to be the best-fit sensor for bacterial RNA in myeloid cells.

There is a renewed interest in TLR7 and TLR8 as key sensors of bacterial viability through single nucleotide polymorphism is linked to better protective immunity in response to a live bacteria vaccine (i.e. BCG). Additionally, TLR8 seems to play a unique role in neonatal immunity as human neonatal plasocytes are only responsive to TLR8 ligands.

A better comprehension of the functional differences between TLR7 and TLR8 should allow the development of more potent, specific and less toxic molecules as stand-alone drugs or adjuvants for the treatment of inflammatory, autoimmune and cancerous diseases.

Species-driven TLR7 and TLR8 differential responses

InvivoGen offers a series of HEK293-derived reporter cells to assess the cellular responses upon stimulation of TLR7 or TLR8, either human or murine. These cell lines individually display distinct response profiles. TLR7 and TLR8 mediate different responses depending on the stimulatory ligand. Moreover, for the same TLR (7 or 8) activated by the same ligand, discrepancies can be observed between the two species (human and mouse).

Human and murine TLR7 or TLR8 reporter cells

- HEK-Blue™ hTLR7 Cells
- HEK-Blue™ mTLR7 Cells
- HEK-Blue™ hTLR8 Cells
- HEK-Blue™ mTLR8 Cells

HEK-Blue™ hTLR7, mTLR7, hTLR8, or mTLR8 cells are derived from the human embryonic kidney (HEK293) cell line. They express the corresponding TLR and TLR7/8 agonists. SEAP levels produced upon TLR7 or TLR8 stimulation can be readily determined by performing the assay in HEK-Blue™ reporter gene. SEAP levels produced upon TLR7 or TLR8 stimulation can be assessed by measuring SEAP activity in the supernatant, using QUANTI-Blue™ detection reagent and reading the OD at 630 nm.

Specific TLR8 inhibitor

- CU-CPT9a NEW

InvivoGen offers CU-CPT9a, a potent and selective inhibitor of TLR8, which binds to and stabilizes the TLR8 dimer in its resting state, thereby preventing its conformational change. This TLR8 antagonist blocks the activation of TLR8 and the subsequent activation of NF-κB without impacting the responses induced by other TLRs, especially the closely related TLR7 (Fig2).

Response profiles

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For more information on TLR7 and TLR8 ligands

www.invivogen.com/tlr78-ligands


Cytosolic sensing of intermediate metabolites of LPS

The ALPK1-TIFA signaling axis is a novel and important cytoplasmic surveillance pathway of pathogenic Gram-negative bacteria, through the sensing of a LPS-intermediary metabolite, ADP-Heptose. To foster research on this pathway, InvivoGen offers a family of products, which include validated knock-out (KO) cells lines and synthetic ADP-Heptose.

The new PAMP on the block

• ADP-Heptose  NEW

InvivoGen has synthesized and purified ADP-Heptose, an intermediary sugar in the biosynthesis of lipopolysaccharide (LPS), an essential component of the outer membrane of Gram negative bacteria. ADP-Heptose is a potent pathogen-associated molecular pattern (PAMP) that binds to the cytosolic pattern recognition receptor (PRR) ALPK1, and triggers a TIFA-dependent pro-inflammatory response through the NF-κB pathway4. ADP-Heptose is delivered to the cytoplasm of host cells by bacterial secretion systems and endocytosed bacteria. Importantly ADP-Heptose can also freely penetrate the host membrane, unlike the other LPS intermediary metabolite, HBP, which not only needs to be enzymatically converted for ALPK1 activation, but also requires a pore-forming agent for delivery4.

InvivoGen’s ADP-Heptose is of the highest quality and has been functionally validated on our HEK-Blue™ Null1-v as well as our HEK-Blue™ KO-ALPK1 and KO-TIFA cell lines (see below).

ALPK1 and TIFA reporter cell lines

• HEK-Blue™ KO-ALPK1 Cells  NEW
• HEK-Blue™ KO-TIFA Cells  NEW
• HEK-Blue™ Null1-v Cells

HEK-Blue™ Null1-v cells derive from the human embryonic kidney (HEK293) cell line, and express a secreted embryonic alkaline phosphatase (SEAP) under the control of an NF-κB/AP1-inducible promoter. Therefore, HEK-Blue™ Null1-v cells are responsive to ADP-Heptose. In the presence of increasing concentrations of ADP-Heptose, these cells produce SEAP in a dose-dependent manner that can be readily monitored using InvivoGen’s SEAP detection reagents, HEK-Blue™ Detection or QUANTI-Blue™ Solution.

In contrast, HEK-Blue™ KO-ALPK1 and HEK-Blue™ KO-TIFA cells are unresponsive to ADP-Heptose, however, they do respond to other NF-κB-inducing cytokines such as human (h)TNF-α. These cells were engineered from the HEK-Blue™ Null1-v cells by stable knock-out (KO) of the ALPK1 and TIFA genes, respectively. These cells are selectable with Zeocin™.

OD (630nm)

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<tr>
<th>ADP-Heptose (µg/ml)</th>
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<tr>
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NF-κB responses of ALPK1 and TIFA reporter cells

Figure 3: NF-κB response in HEK293-derived ALPK1 and TIFA reporter cells. HEK-Blue™ Null1-v, KO-ALPK1, and KO-TIFA cells were incubated with increasing concentrations of (A) ADP-Heptose (0-100 µg/ml) and (B) human (h)TNF-α (0-100 ng/ml) in HEK-Blue™ Detection, a cell culture medium for SEAP detection. After overnight incubation, the NF-κB response was assessed by measuring the activity of SEAP in the supernatant. OD was read at 630 nm.

Other products you may need

<table>
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<td>ADP-Heptose</td>
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<td>HEK Blue™ KO-TIFA Cells</td>
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<tr>
<td>HEK Blue™ Null1-v Cells</td>
<td>3-7 x 10⁶ cells</td>
<td>hkb-null1v</td>
</tr>
</tbody>
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Prevention of contamination in primary cell cultures

Primocin™

Primary cell cultures face a constant threat of microbial contamination both from the original source and the surrounding environment. To help protect your cells InvivoGen offers Primocin™, a broad-spectrum antibiotic formulation that is gentle on your cells but lethal to the microbes.

- **Broad spectrum:** Kills bacteria, mycoplasma, and fungi
- **Safe:** Non-toxic to primary cells
- **Trusted:** Frequently cited in the literature

Primocin™ is an antibiotic formulation designed to offer complete protection to primary cell cultures from microbial contamination. It contains compounds that block DNA and protein synthesis in Gram-positive and Gram-negative bacteria, as well as mycoplasmas. Additionally, it contains a compound that specifically targets fungi by disrupting ionic exchange through the cell membrane. Primocin™ is non-toxic to primary cells when used at the recommended concentration.

**Use of Primocin™ in primary cell cultures**

Primocin™ is frequently cited in the literature for use in the protection of a number of different primary cell cultures.

- **Differentiated cells**
  Primocin™ has been shown to be important in the isolation and culturing of several differentiated human and murine cell types. These include fibroblasts, astrocytes, and NK cells, and from different sources such as peripheral blood mononuclear cells (PBMCs) and extracted tissues.

- **Pluripotent stem cells**
  In the development of long-term cultures of induced human pluripotent stem cells (iPSCs), Primocin™ aids in the protection against bacterial and mycoplasma infection. It has been defined as a “critical addition” used throughout the culturing and reprogramming of stem cells.

- **Organoid cultures**
  In the emerging and exciting field of 3D cell culture and organoid growth, Primocin™ has shown great importance in providing essential protection during their development. It is included routinely in the growth of colon epithelial and carcinoma organoids as well as bladder, breast, and prostate cancer organoids.

Around the world, researchers trust Primocin™ to protect their precious primary cell cultures from damaging, time-consuming, and costly microbial contamination.


Where does contamination come from?

There are a number of sources of contamination including lab operators and dirty equipment (waterbaths, incubators, and glassware). Unfortunately, in the isolation of cells from both animal and human tissue, contamination from commensal flora and/or subclinical infections is common. InvivoGen provides highly referenced antibiotic cocktails to both prevent and eradicate a wide range of microbes including bacteria, mycoplasma, and fungi.

Protect your cells with InvivoGen

No matter the type of contamination you want to prevent or eradicate, InvivoGen has the solution.

- **Normocin™** Anti-microbial agent #ant-nr-1
- **Plasmocin™** Anti-mycoplasma agent #ant-mpt-1
- **Fungin™** Anti-fungal agent #ant-fn-1

www.invivogen.com/cell-culture-contamination

**PRODUCT** | **QUANTITY** | **CAT. CODE**
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Primocin™ | 500 mg (10 x 1 ml) | ant-pm-1

www.invivogen.com/primocin

InvivoGen

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