InvivoGen insight

SUMMARY :

REVIEW

TLR7 & TLR8: fraternal twins

PRODUCTS

Species-driven TLR7 and TLR8 responses

- HEK-Blue™ hTLR7 Cells
- HEK-Blue™ mTLR7 Cells
- HEK-Blue™ hTLR8 Cells
- HEK-Blue™ mTLR8 Cells
- CU-CPT9a: TLR8 specific inhibitor

Cytosolic sensing of intermediate metabolites of LPS

- ADP-Heptose
- HEK-Blue™ KO-ALPK1 Cells
- HEK-Blue™ KO-TIFA Cells
- HEK-Blue™ Null1-v Cells

Preventing contamination of primary cell cultures

- Primocin™

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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.

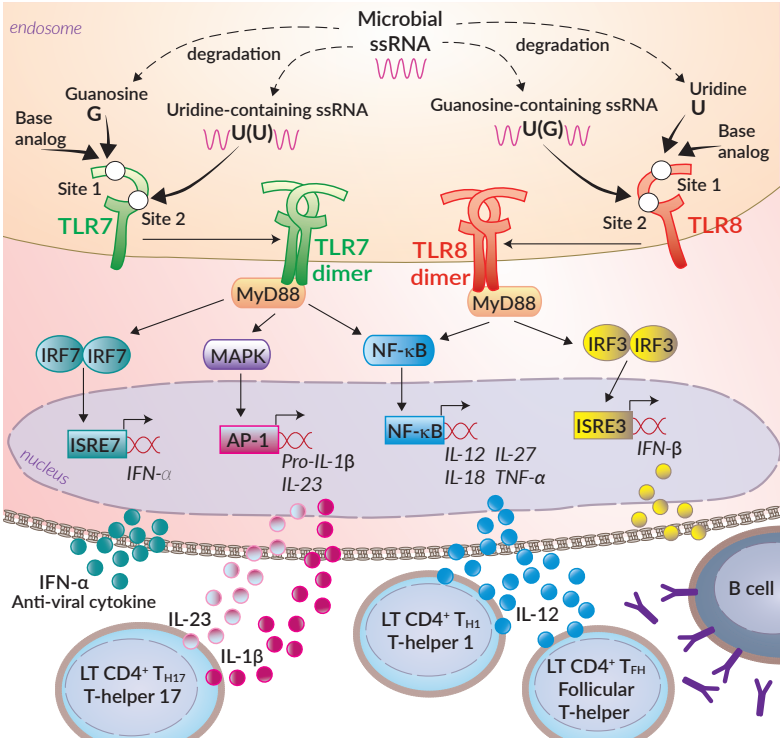
The endosomal distribution of TLR7 and TLR8 allows them to scan for the presence of microbial RNA in the phagocytic cargo. Their activation leads to NF-κB-, AP1- and IRF-mediated production of type I IFNs (IFN-α/β) and pro-inflammatory cytokines[1]. Structural analyses have revealed that both TLR7 and 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[2,3]. 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[2,3]. Thus, TLR7 and TLR8 appear to sense distinct RNA-degradation products rather than full-length ssRNAs[3].

TLR8 has been less studied than TLR7 as it was initially thought to be non-functional in mice[4]. Of note, this does not hold true when using TL8-506, an analog of the synthetic agonist VX-2337 (see inside). Further, TLR13 has been suggested as a murine TLR8 homolog[5]. Thus, findings regarding mouse TLR7 and TLR8 are not transposable to their human counterparts[4,6]. 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[7].

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[1]. TLR8 is absent from pDCs and B cells, and is highly expressed by myeloid cells[1]. 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[1,6]. However, in monocytes, TLR7 and TLR8 activation induces the expression of T_H17- and T_H1-skewing cytokines, IL-1β and IL-12, respectively[8]. Upon bacterial infection, TLR7 drives IFN-α production by pDCs, but its role in myeloid cells remains obscure[6]. On the other hand, TLR8 seems to be the 'best-fit' sensor for bacterial RNA in myeloid cells[6,7].

There is converging evidence for TLR8 to be the missing link between empirical use of live attenuated microbes in vaccines and the known necessity for T_H1- and T_{FH}-driven humoral immunity to reach superior vaccine efficiencies. Live bacteria or bacterial RNA, but not dead bacteria, induce the TLR8-dependent production of IL-12 by human monocytes, thereby promoting T_{FH} differentiation[6]. Moreover, epidemiological analyses indicate that the gain-of-function of human TLR8 through single nucleotide polymorphism is linked to better protective immunity in response to a live bacteria vaccine (i.e. BCG)[7]. Lastly, TLR8 seems to play a unique role in neonatal immunity as human neonatal phagocytes are only responsive to TLR8 ligands[9].

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.



1. Georg P. & Sander L.E., 2019. Innate sensors that regulate vaccine responses. Curr. Op. Immunol. 59:31. **2. Zhang Z. *et al.*, 2018.** Structural analyses of Toll-like receptor 7 reveal detailed RNA sequence specificity and recognition mechanism of agonistic ligands. Cell Rep. 25:3371. **3. Tanji H. *et al.*, 2015.** Toll-like receptor 8 senses degradation products of single-stranded RNA. Nat. Struct. Mol. Biol. 22:109. **4. Heil F. *et al.*, 2004.** Species-specific recognition of single-straned RNA via Toll-like receptor 7 and 8. Science. 303:1526. **5. Choo M.K. *et al.*, 2017.** TLR sensing of bacterial spore-associated RNA triggers host immune responses with detrimental effects. J. Exp. Med. 214:1297. **6. Eigenbrod T. & Dalpke A.H., 2015.** Bacterial RNA: an underestimated stimulus for innate immune respones. J. Immunol. 195:411. **7. Ugolini M. *et al.*, 2018.** Recognition of microbial viability via TLR8 drives TFH cell differentiation and vaccine responses. Nat. Immunol. 19:386. **8. De Marcken M. *et al.*, 2019.** TLR7 and TLR8 activate distinct pathways in monocytes during RNA virus infection. Sci. Signaling. 12:eaaw1347. **9. Levy O. *et al.*, 2006.** Unique efficacy of Toll-like receptor 8 agonists in activating human neonatal antigen-presenting cells. Blood. 108:1284.

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 an NF-κB/AP1-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. SEAP levels produced upon TLR7 or TLR8 stimulation can be readily determined by performing the assay in HEK-Blue™ Detection medium. These cells are selectable with Blasticidin and Zeocin™.

Response profiles	R848	Imiquimod	ssRNA40/ LyoVec™	TL8-506
	TLR7/8	TLR7	TLR8	TLR8
HEK-Blue™ hTLR7 cells	■	■		
HEK-Blue™ mTLR7 cells	■	■	*	*
HEK-Blue™ hTLR8 cells	■		■	■
HEK-Blue™ mTLR8 cells	**		**	■

* mTLR7 and mTLR8 share strong homology and may have evolved to detect a broad overlapping range of ligands.
** The addition of poly(dT) rescues the responses to ssRNA40, and various TLR7/8 agonists[1].

Human and mouse TLR7- or TLR8-induced responses

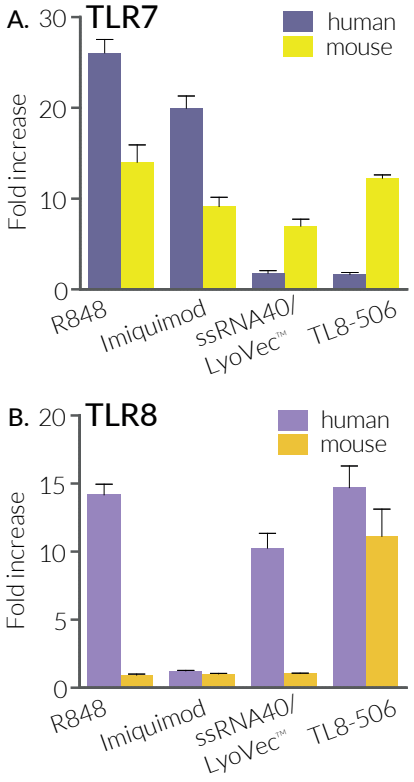


Figure 1: TLR7 and TLR8 induction in HEK293-derived reporter cells. HEK-Blue™ hTLR7 or mTLR7 (A), and HEK-Blue™ hTLR8 or mTLR8 (B) were cultured in HEK-Blue™ Detection medium with 1 µg/ml R848 (TLR7/8 agonist), 3 µg/ml Imiquimod (TLR7 agonist), 5 µg/ml ssRNA40/LyoVec™ (referred as human TLR8 agonist), or 1 µg/ml TL8-506 (TLR8 agonist, VTX-2337 analog). After 24h incubation, TLR7- or TLR8-induced NF-κB/AP1 responses were assessed by measuring SEAP levels in the supernatant by reading the OD at 630 nm. OD fold increase over non-induced cells is shown.

1. Liu J. *et al.* 2009. A five-amino-acid motif in the undefined region of the TLR8 ectodomain is required for species-specific ligand recognition. Mol. Immunol. 47:1083..

www.invivogen.com/hek-blue-tlr

Specific TLR8 inhibitor

- CU-CPT9a NEW

InvivoGen offers CU-CPT9a, a potent and selective inhibitor of TLR8[2,3] (Fig2). CU-CPT9a 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[2] (Fig2).

www.invivogen.com/cucpt9a

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue™ hTLR7 Cells	3-7 x 10^6 cells	hkb-htlr7
HEK-Blue™ mTLR7 Cells	3-7 x 10^6 cells	hkb-mtlr7
HEK-Blue™ hTLR8 Cells	3-7 x 10^6 cells	hkb-htlr8
HEK-Blue™ mTLR8 Cells	3-7 x 10^6 cells	hkb-mtlr8
R848 (Resiquimod)	500 µg	tlrl-r848
Imiquimod (R837)	500 µg	tlrl-imqs
ssRNA40/LyoVec™	4 x 25 µg	tlrl-lrna40
TL8-506	500 µg	tlrl-tl8506
CU-CPT9a	10 mg	inh-cc9a

CU-CPT9a inhibition of TLR8-induced responses

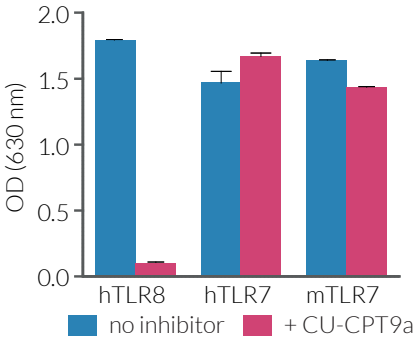


Figure 2: Specific inhibition of human TLR8 by CU-CPT9a. HEK-Blue™ hTLR8, hTLR7 or mTLR7 cells were incubated with 1 µM CU-CPT9a for 3 hours before adding 10 µg/ml of R848 (TLR7/8 agonist). After overnight incubation, NF-κB activity was assessed by measuring SEAP activity in the supernatant, using QUANTI-Blue™ Solution detection reagent and reading the OD at 630 nm.

2. Zhang, S. *et al.* 2018. Small-molecule inhibition of TLR8 through stabilization of its resting state. Nat. Chem. Biol. 14(1):58-64. 3. Moen, S.H. *et al.* 2019. Human Toll-Like Receptor 8 (TLR8) is an important sensor of pyogenic bacteria and is attenuated by cell surface TLR signaling. Front. Immunol. 10. 1209.

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-intermediatry 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

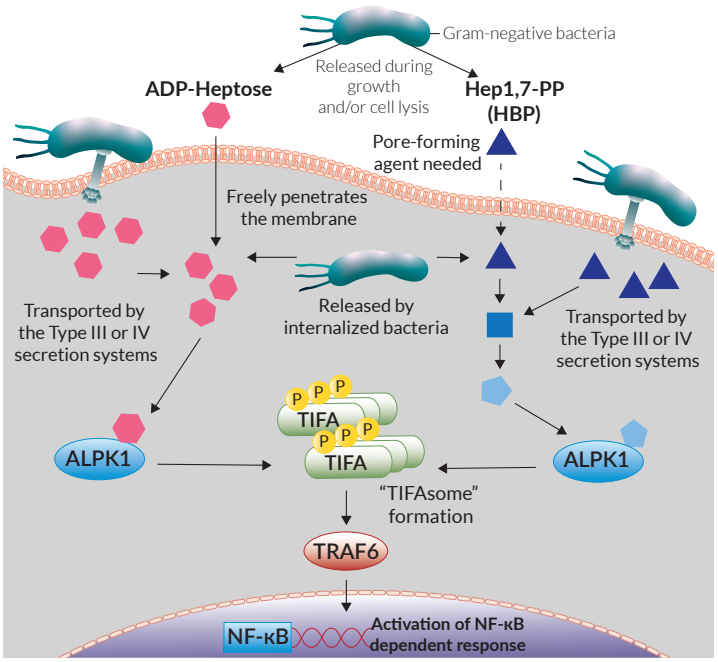
- ADP-Heptose NEW

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 pathway[4]. 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 delivery[4].
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*).

4. Zhou, P. *et al.* 2018. Alpha-kinase 1 is a cytosolic innate immune receptor for bacterial ADP-heptose. Nature 561, 122-126.

www.invivogen.com/adp-heptose

ADP-Heptose induced ALPK1-TIFA signaling



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™.

www.invivogen.com/ko-alpk1-tifa-cells

NF-κB responses of ALPK1 and TIFA reporter cells

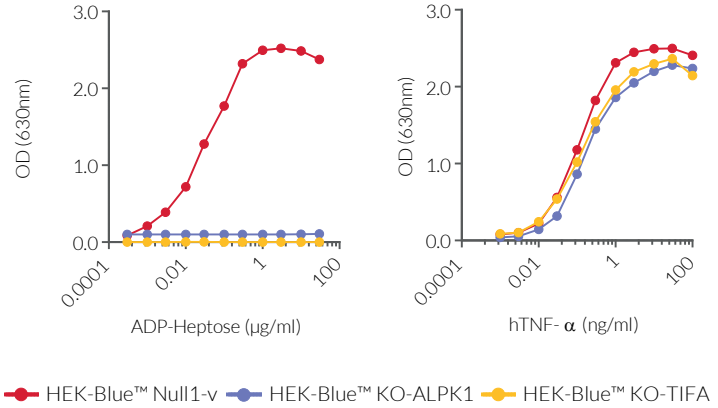


Figure 3: NF-κB response in HEK293-derived ALPK1 and TIFA reporter cells. HEK-Blue™ Null1-v, KO-ALPK1, and KO-TIFA cells were incubated with increasing concentrations of (A) ADP-Heptose (0-100 µg/ml) and (B) human (h)TNF-α (0-100 ng/ml) in HEK-Blue™ Detection, a cell culture medium for SEAP detection.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

PRODUCT	QUANTITY	CAT. CODE
ADP-Heptose	250 µg	tlrl-adph
HEK Blue™ KO-ALPK1 Cells	3-7 x 10^6 cells	hkb-koalpk
HEK Blue™ KO-TIFA Cells	3-7 x 10^6 cells	hkb-kotifa
HEK Blue™ Null1-v Cells	3-7 x 10^6 cells	hkb-null1v

Other products you may need

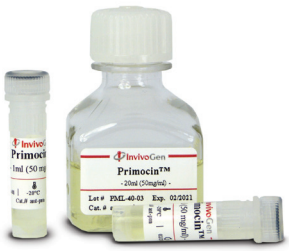
HEK-Blue™ Detection	SEAP detection medium	#hb-det2
QUANTI-Blue™	SEAP detection reagent	#rep-qbs
Rec. hTNF-α	Recombinant human cytokine	#rcyc-htnfa
Zeocin™	Selective antibiotic	#ant-zn-1

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[1], astrocytes[2], and NK cells[3], 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[4].

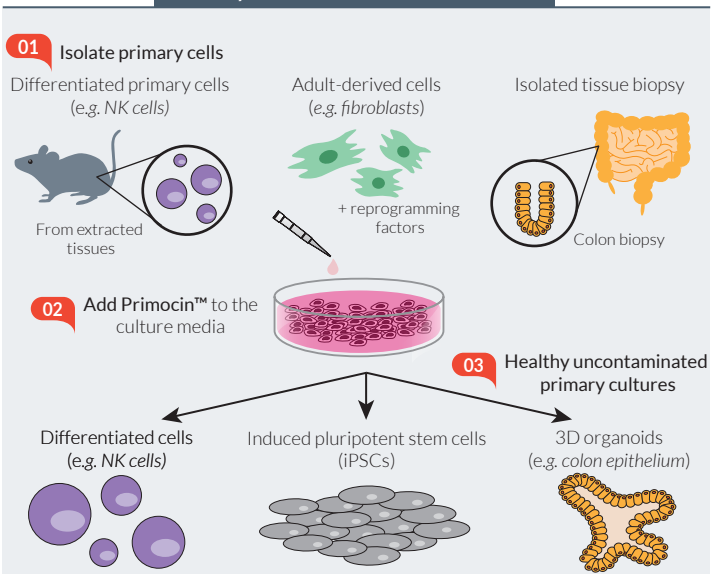
- Organoid cultures

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[5] as well as bladder, breast, and prostate cancer organoids[6].

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

1. **Ferrer-Mayorga, G. *et al.* 2019.** Vitamin D and Wnt3A have additive and partially overlapping modulatory effects on gene expression and phenotype in human colon fibroblasts. Sci Rep 9, 8085. 2. **Grabner, G.F. *et al.* 2016.** Deletion of Monoglyceride Lipase in Astrocytes Attenuates Lipopolysaccharide-induced Neuroinflammation. J Biol Chem 291, 913-923. 3. **Garcia-Beltran, W.F. *et al.* 2016.** Open conformers of HLA-F are high-affinity ligands of the activating NK-cell receptor KIR3DS1. Nat Immunol 17, 1067-1074. 4. **Park, S. *et al.* 2018.** Generation of Human Induced Pluripotent Stem Cells Using a Defined, Feeder-Free Reprogramming System. Curr Protoc Stem Cell Biol 45, e48. 5. **Urbischek, M. *et al.* 2019.** Organoid culture media formulated with growth factors of defined cellular activity. Sci Rep 9, 6193. 6. **Xu, H. *et al.* 2018.** Organoid technology and applications in cancer research. J Hematol Oncol 11, 116.

Primary cell cultures and Primocin™



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
Primocin™	500 mg (10 x 1 ml)	ant-pm-1

www.invivogen.com/primocin

InvivoGen

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