InvivoGen insight

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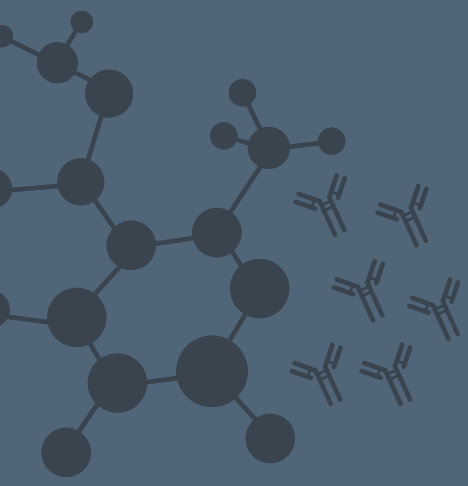

InvivoGen
www.invivogen.com
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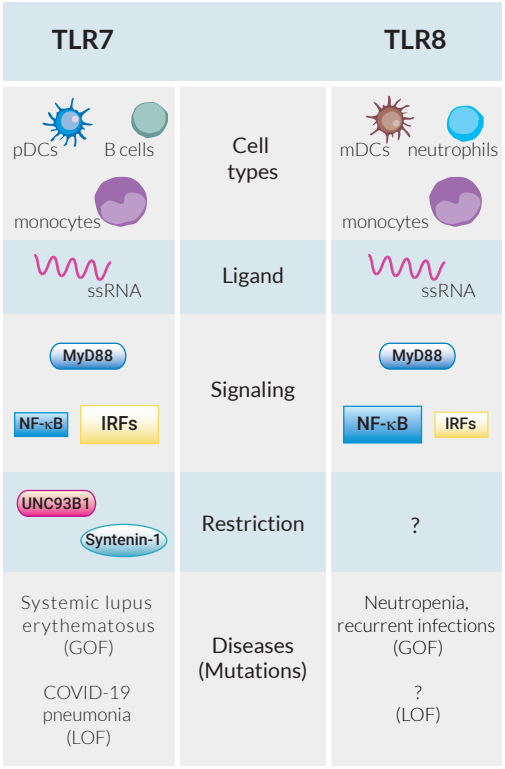
TLR7 and TLR8: same, same... but different!

The innate immune system is armed with a limited set of germline-encoded pattern recognition receptors (PRRs) capable of sensing a tremendous variety of potential pathogens[1]. Among these PRRs, two phylogenetically and structurally highly related Toll-like receptors, TLR7 and TLR8, recognize the same ligand, single-stranded (ss) RNA[2]. Upon stimulation, they both induce the NF-κB and IRF pathways via MyD88, activating multiple immune cells (e.g. T cells), thus bridging the innate and adaptive immune response[3]. Based on these observations, it is tempting to speculate a redundant role of these two receptors, as they also share other functional homologies[1].

Despite all evidence to the contrary, TLR7 and TLR8 have evolved under strong selection, suggesting their essential and unique functions in host survival[4]. They differ in cell type-specific expression with TLR7 found in plasmacytoid dendritic cells (p)DCs and B cells, and TLR8 in myeloid (m)DCs and neutrophils. Among immune cells, only monocytes co-express both receptors[5]. While pDCs rapidly secrete large amounts of IFN-α upon IRF activation, mDCs are more involved in the NF-κB-dependent production of pro-inflammatory cytokines (e.g. IL-12, TNF-α)[5]. Moreover, both receptors contribute differently to diseases. Recently, TLR7-deficiency has been reported as a genetic mediator for severe COVID-19 pneumonia due to impaired SARS-CoV-2 detection and type I IFN production[6]. To date, no disease has yet been attributed to TLR8 loss-of-function[7]. Whether it triggers no phenotype, is lethal, or the TLR8-specific pathogen is extinct and/or yet undiagnosed remains to be elucidated. Regarding hyperactivation, improper TLR7 stimulation by self-RNA has been linked to systemic lupus erythematosus (SLE), a polygenic autoimmune disorder, characterized by the presence of selfnucleic antigens and autoantibodies[8]. Recently, the first monogenic form of SLE caused by a gain-of-funtion (GOF) mutation in TLR7 has been described[9]. In contrast, TLR8 GOF causes a more complex phenotype with congenital neutropenia (low level of neutrophils) and recurrent infections, but surprisingly without autoimmunity[7].

In order to distinctively dissect TLR7- and TLR8-dependent signaling, InvivoGen has developed a unique collection of HEK- and THP-1-derived cell lines, allowing the simultaneous study of the two major signaling pathways linked to TLR7/8, the NF-κB and IRF pathways. Unexpectedly, generating functional *TLR7*-expressing clones was extremely difficult: Whereas stable TLR7 expression was easily achieved, it was impossible to induce TLR7 activation, suggesting either missing co-factors or an underlying negative regulation of TLR7. Interestingly, this was not observed in TLR8 overexpressing cells.



A growing body of literature has highlighted the role of two key adaptor proteins UNC93B1 and syntenin-1 controlling the intracellular location, stability and signal duration of endosomal TLRs[10-11]. The chaperone protein UNC93B1 mediates TLR-trafficking from the endoplasmic reticulum to the endosome. It specifically limits TLR7 translocation by favoring the transport of other endosomal TLRs[10]. Moreover, it remains associated to TLR7 in the endosome to stabilize it and to recruit syntenin-1. Upon TLR7 activation, syntenin-1 terminates TLR7 signaling by sorting it into intraluminal vesicles, thereby drastically reducing the half-life of functional TLR7[11]. Various mutations in UNC93B1 were described to abolish these modulatory roles, and unleash TLR7[12]. Indeed, by introducing a mutant version of UNC93B1 in our TLR7-overexpressing cells, we were able to overcome the restrictive mechanism and obtain stable TLR7 signaling. The question remains why TLR7 and not TLR8 requires this strong UNC93B1-mediated regulation. Given they harbor the potential of autoreactivity to ssRNA, both their sensing certainly has to be tightly controlled. So what is restricting TLR8?

Overall, our journey of generating these cell lines perfectly illustrates the underlying complexity of TLR7/8 activity. InvivoGen's new cell lines will support scientists on their way to unravel the many mysteries surrounding TLR7 and TLR8, as well as tailor new therapeutic approaches for infectious and autoimmune diseases.

1. Lind NA, *et al.*, Nat Rev Immunol. 2022 Apr;22(4):224-235. **2. Heil F, *et al.*,** Science. 2004 Mar 5;303(5663):1526-9. **3. Sun H, *et al.*,** Biomark Res. 2022 Dec 7;10(1):89.. **4. Barreiro LB, *et al.*,** PLoS Genet. 2009 Jul;5(7): e1000562. **5. Martínez-Espinoza I, *et al.*,** Pathogens. 2022 Jan 22;11(2):134. **6. Asano T, *et al.*,** Sci Immunol. 2021 Aug 19;6(62):eabl4348. **7. Aluri J, et al., Blood.** 2021 May 6;137(18):2450-2462. **8. Celhar T, *et al.*,** Immunol Res. 2012 Sep;53(1-3):58-77. **9. Brown GJ, *et al.*,** Nature. 2022 May;605(7909):349-356. **10. Majer O, *et al.*,** Nature. 2019 Nov;575(7782):366-370. **11. Pelka K, *et al.*,** Immunity. 2018 May 15;48(5):911-922.e7. **12. Fukui R, *et al.*,** J Exp Med. 2009 Jun 8;206(6):1339-50.

TLR7 & TLR8 Dual™ reporter cell lines

InvivoGen offers a series of THP-1-derived cell lines to facilitate the study of human (h)TLR7 and hTLR8, two closely-related endosomal TLRs. These human monocytic reporter cells are designed to monitor the NF-κB and IRF responses downstream TLR7 and/or TLR8. To ensure the screening of specific ligands, the cell lines feature expression and/or knockout (KO) of hTLR7 and hTLR8.

- **THP1-Dual™ hTLR7 Cells** NEW
- **THP1-Dual™ hTLR7 KO-TLR8 Cells** NEW
- **THP1-Dual™ hTLR8 Cells** NEW
- **THP1-Dual™ KO-TLR8 Cells**

Key Features

- Dual reporter system: NF-κB-SEAP and IRF-Lucia
- Stable expression of TLR7, TLR8, or TLR7 and TLR8
- Highly responsive to TLR7 and TLR8 specific ligands

THP-1 monocytes offer a physiological background for innate immunity studies as they express various PRRs and PRR-related genes involved in the signaling cascades[1]. Of note, they endogenously express TLR8, but not TLR7[1,2]. InvivoGen's **THP1-Dual™-derived cells** feature two inducible reporter genes for SEAP (secreted embryonic alkaline phosphatase) and Lucia luciferase to monitor the NF-κB and IRF pathways, respectively. SEAP and Lucia activities can be readily assessed in the supernatant using QUANTI-Blue™ Solution and QUANTI-Luc™ 4 detection reagents.

In **THP1-Dual™ hTLR7** and **THP1-Dual™ hTLR7 KO-TLR8 cells**, the co-expression of TLR7 and a mutated (mut) version of the chaperone protein UNC93B1 allows NF-κB and IRF responses upon stimulation with TLR7- and TLR7/8-specific agonists. The combined use of both cell lines ensures the distinction between TLR7- and TLR8-mediated activation. Interestingly, the KO of endogenous *TLR8* increases the IRF-response to R848, a TLR7/8 agonist *(Fig. 1)*.

In **THP1-Dual™ hTLR8 cells**, the overexpression of hTLR8 strongly increases the NF-κB and IRF responses to TLR7/8 and TLR8 agonists, compared to the THP1-Dual™ parental cells. As expected, the KO of endogenous *TLR8* in **THP1-Dual™ KO-TLR8 cells** abrogates these responses *(Fig. 1)*.

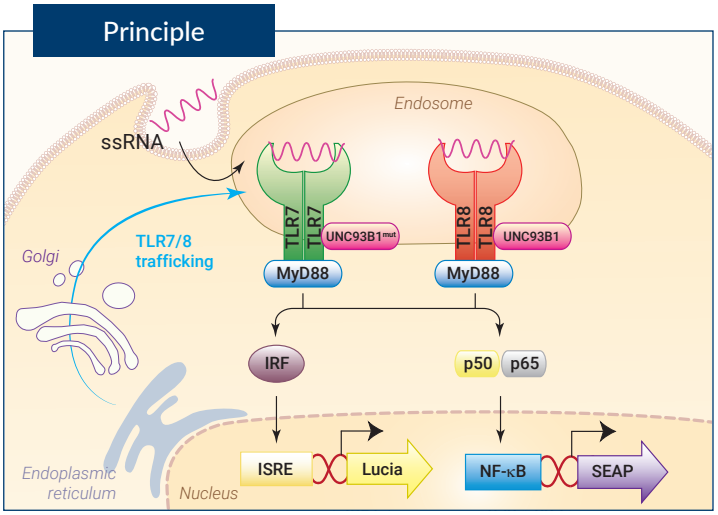
1. Hornung V. *et al.*, 2002. Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J Immunol.; 168(9):4531-7. 2. Majer, O., *et al.* 2019. UNC93B1 recruits syntenin-1 to dampen TLR7 signalling and prevent autoimmunity. Nature 575, 366–370.

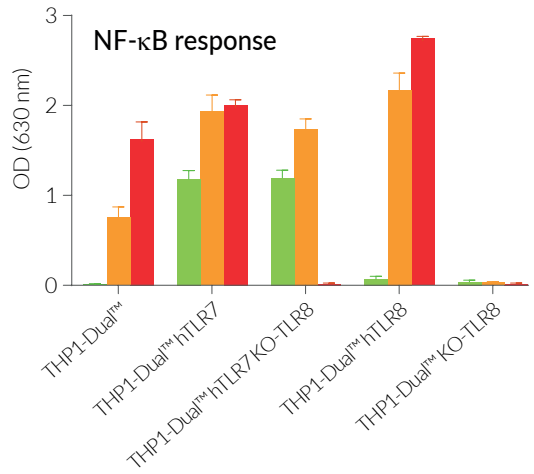
PRODUCTS	QTY	CAT. CODE
THP1-Dual™ hTLR7 Cells	3-7 x 10^6 cells	thpd-htlr7
THP1-Dual™ hTLR7 KO-TLR8 Cells	3-7 x 10^6 cells	thpd-htlr7-ko8
THP1-Dual™ hTLR8 Cells	3-7 x 10^6 cells	thpd-htlr8
THP1-Dual™ KO-TLR8 Cells	3-7 x 10^6 cells	thpd-kotlr8

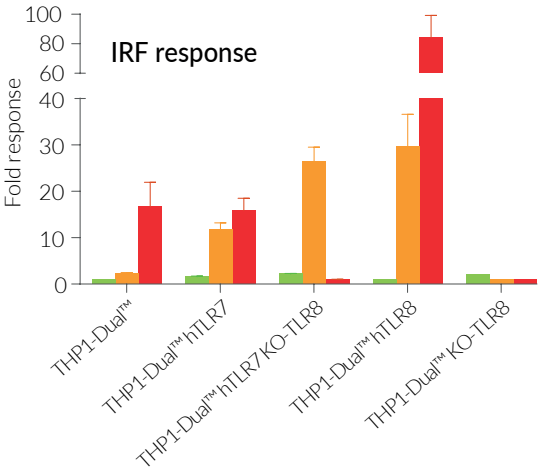
RELATED PRODUCTS

- **THP1-Dual™ cells:** Parental reporter cell line (thpd-nfis)
- **QUANTI-Blue™:** SEAP detection reagent, liquid (rep-qbs)
- **QUANTI-Luc™ 4:** Luciferase detection reagent, liquid (rep-qlc4lg1)

www.invivogen.com/thp1-dual-htlr7-htlr8







Imiquimod (TLR7) R848 (TLR7/8) TL8-506 (TLR8)

Figure 1. NF-κB and IRF responses in TLR7/8 expressing THP1-Dual™-derived cells. Cells were incubated for 24h with Imiquimod (10 µg/ml), R848 (10 µg/ml), and TL8-506 (1 µg/ml). After 24h incubation, the NF-κB-induced SEAP activity was assessed using QUANTI-Blue™ Solution. Data are shown as optical density (OD) at 630 nm (mean ± SEM). IRF-induced Lucia luciferase activity was assessed using QUANTI-Luc™. Data are shown in fold response over non-induced cells (mean + SEM).

TLR7/8 agonists and inhibitors

InvivoGen provides a comprehensive choice of TLR7/8 agonists specific for either TLR7 or TLR8, or both. To discriminate between specific activation of TLR7 or TLR8, two potent antagonists are also available. These high-quality ligands are functionally-tested.

TLR7/8 agonists

- **Imiquimod** (TLR7-specific)
- **R848** (TLR7- and TLR8-specific)
- **TL8-506** (TLR8-specific)

Imidazoquinoline derivatives (IMDs) and related compounds are synthethic agonists of TLR7/8, many of which are being tested to treat infections as well as malignant tumors owing to their adjuvant properties. **Imiquimod** is TLR7-specific and is the only IMD approved by the FDA to date. **R848** (aka Resiquimod) is a TLR7 and TLR8 agonist. This IMD is more potent than Imiquimod in stimulating TLR7, but has failed due to clinical toxicity. **TL8-506** is a benzoazepine compound, an analog of VTX-2337 currently in clinical trials. It is a TLR8-specific agonist which is more potent than R848. Importantly, it is the only synthetic base analog described to activate mouse TLR8 *(Fig. 1)*.

We also offer synthetic single stranded RNAs which function as human and mouse TLR8, as well as mouse TLR7 agonists.
Check our website to learn more. www.invivogen.com/tlr7-8-ssrna

TLR7/8 inhibitors

- **M5049** (TLR7- and TLR8-specific) **NEW**

M5049 (also known as Enpatoran) is a dual and selective inhibitor of TLR7 and TLR8[1]. This drug is currently under investigation as an oral treatment for COVID-19 and lupus erythematosus. M5049 efficiently inhibits human (h)TLR7, mouse (m)TLR7, and hTLR8, but not mTLR8. It has no impact on other endosomal TLRs, *i.e.* TLR3 and TLR9 *(Fig. 2)*.

M5049 binds and stabilizes the hTLR8 dimer in its resting (inactive) state, antagonizing the binding of any TLR8 ligands[1]. It has been suggested to act similarly for TLR7. This mode of action has also been described for CU-CPT9a, a specific inhibitor of hTLR8 and mTLR8.

1. Vlach J. *et al.*, 2020. Discovery of M5049: a novel selective Toll-Like Receptor 7/8 inhibitor for treatment of autoimmunity. J Pharmacol Exp Ther. 376:397.

PRODUCTS	QTY	CAT. CODE
Imiquimod (R837)	500 µg	tlrl-imqs
R848 (Resiquimod)	500 µg	tlrl-r848
TL8-506	500 µg	tlrl-tl8506
M5049	5 mg	inh-m5049

RELATED PRODUCTS

- **HEK-Blue™ hTLR7** (hkb-htlr7)
- **HEK-Blue™ hTLR8** (hkb-htlr8)
- **HEK-Blue™ mTLR8** (hkb-mtlr8)
- **HEK-Blue™ mTLR7** (hkb-mtlr7)
- **CU-CPT9a:** selective inhibitor of hTLR8 and mTLR8 (inh-cc9a)

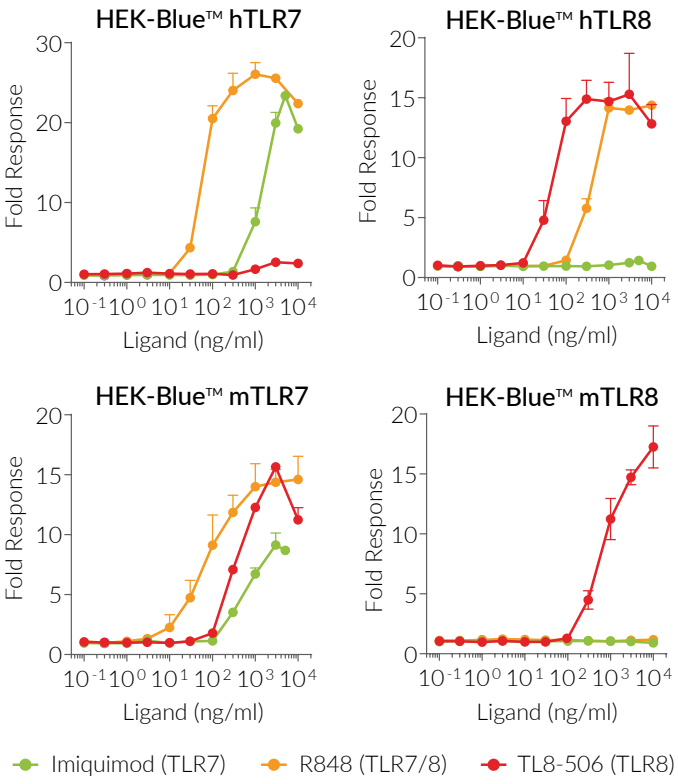


Figure 1. Activation of human and mouse TLR7 and TLR8 in HEK-Blue™-derived cell lines. HEK-Blue™ cells expressing hTLR7, mTLR7, hTLR8, or mTLR8 were cultured with increasing concentrations of Imiquimod, R848, or TL8-506. After overnight incubation, TLR7- and TLR8-induced NF-κB response was assessed by measuring SEAP activity in the supernatant, using QUANTI-Blue™ Solution. Data are shown in fold response over non-induced cells (mean + SEM).

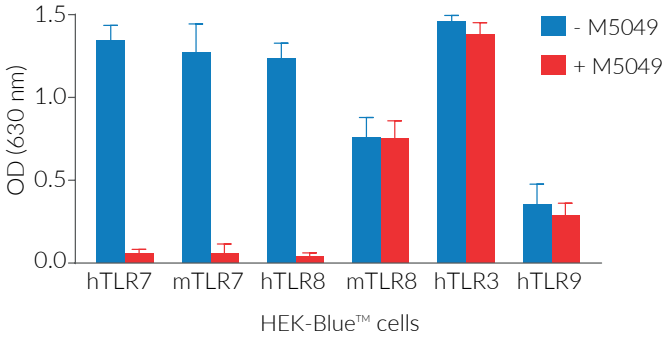


Figure 2. Specific inhibition of TLR7 and TLR8 by M5049. HEK-Blue™ cells expressing human (h)TLR7, mouse (m)TLR7, hTLR8, mTLR8, hTLR3, hTLR4, or hTLR9 were incubated with M5049 (1 µM). After 3 hours of incubation, the following ligands were added: R848 30 ng/ml (for hTLR7) or 100 ng/ml (for mTLR7), TL8-506 30 ng/ml (for hTLR8) or 300 ng/ml (for mTLR8), Poly(I:C) HMW 50 ng/ml (for hTLR3), LPS-EK 1 ng/ml (for hTLR4), and ODN 2006 500 ng/ml (for hTLR9). After overnight incubation, the neutralizing activity of M5049 was determined by measuring the reduction of SEAP production using the HEK-Blue™ detection reagent. Data are shown as optical density (OD) at 630 nm (mean +SEM).

www.invivogen.com/tlr78-ligands

QUANTI-Blue™ Solution

InvivoGen offers a liquid formulation of QUANTI-Blue™ Solution for the rapid detection of secreted embryonic alkaline phosphatase (SEAP), by observing a simple color change from pink to purple/blue in live-cell supernatants. This highly sensitive solution has been optimized for use in 96-well plates (standard procedure) and in 1536-well plates (high-throughput screening procedure).

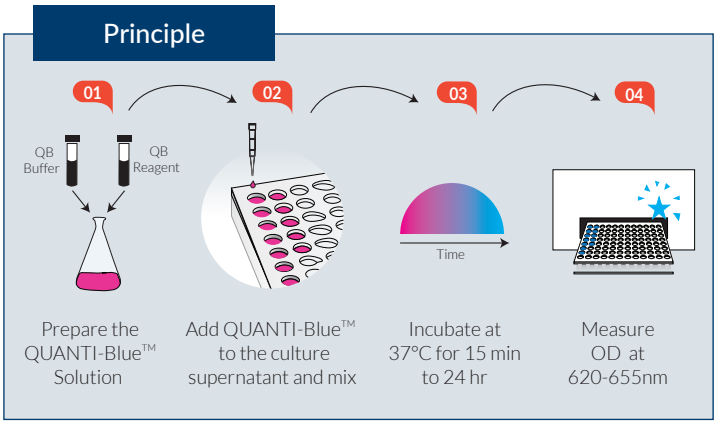
Key Features

- Convenient, stable, and cost-effective
- Short hands-on time: <10 min
- Highly sensitive and wide dynamic range
- Suitable for High-throughput screenings (HTS)

InvivoGen's liquid formulation of QUANTI-Blue™ offers a highly sensitive and rapid detection of secreted embryonic alkaline phosphatase (SEAP), by observing a simple color change from pink to purple/blue.

QUANTI-Blue™ Solution is concentrated (100x) and is therefore adaptable to your needs. It has been optimized for use at either 1x or up to 10x, depending on your sample size. Moreover, it can be added directly to the cells in culture plates, making it ideal for HTS.

Check our website to learn more about our wide collection of SEAP reporter cell lines. www.invivogen.com/reporter-cells

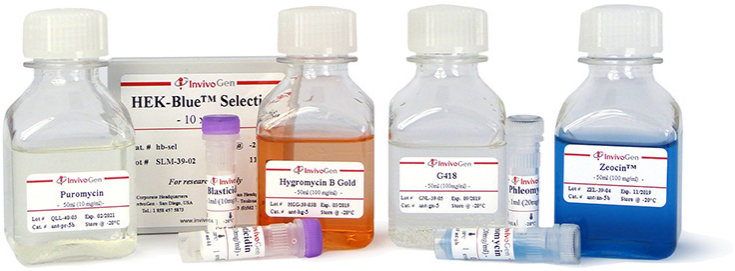


PRODUCTS	QTY	CAT. CODE
QUANTI-Blue™ Solution	5 ml (100 X)	rep-qbs
	10 ml (100 X)	rep-qbs2
	20 ml (100 X)	rep-qbs3

www.invivogen.com/reporter-detection

Selective Antibiotics

InvivoGen is a leader in the production of selection antibiotics. Our state-of-the-art facilities allow us to produce large quantities of high quality, endotoxin-free antibiotics with purity levels exceeding 95%. These selective antibiotics are available as sterile-filtered solutions for customer convenience and validated for cell culture usage.



Key Features

- Ready-to-use sterile filtered
- Endotoxin-free
- Each lot is functional tested

InvivoGen offers a range of cell-culture tested antibiotics to ensure artifact-free selection of transfected mammalian cells. These antibiotics are sterile and endotoxin-free to avoid the deleterious effects of bacterial endotoxins on transfected cells. They are functionally validated through rigorous physico-chemical, microbiological and cellular testing.

www.invivogen.com/selective-antibiotics

PRODUCTS	QTY	CAT. CODE
Blasticidin	100 mg (10 x 1 ml)	ant-bl-1
G418 (Geneticin)	1 g (10 x 1 ml)	ant-gn-1
HEK-Blue™ Selection	10 x 1ml	hb-sel
Hygromycin B Gold	1 g (10 x 1 ml)	ant-hg-1
Phleomycin	100 mg (10 x 1 ml)	ant-ph-1
Puromycin	100 mg (10 x 1 ml)	ant-pr-1
Zeocin®	1 g (10 x 1 ml)	ant-zn-1

InvivoGen

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