InvivoGen insight

NOVEMBER 2018

SUMMARY :

REVIEW

AhR's key role in the intestinal microbiota and immunity

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QUANTI-Blue™ Solution

InvivoGen

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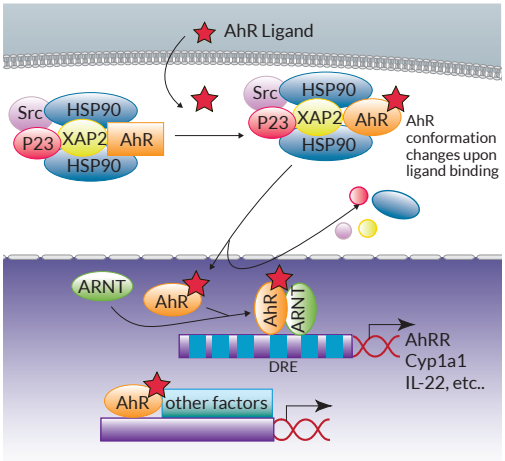
AhR's key role in the intestinal microbiota and immunity

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcriptional factor widely expressed among immune, epithelial, endothelial and stromal cells in barrier tissues[1]. While historically studied in the context of chemical pollutants such as dioxin, AhR was more recently revealed as a central sensor of a wider range of environmental cues, ensuring intestinal homeostasis between the host and gut microbiota[1].

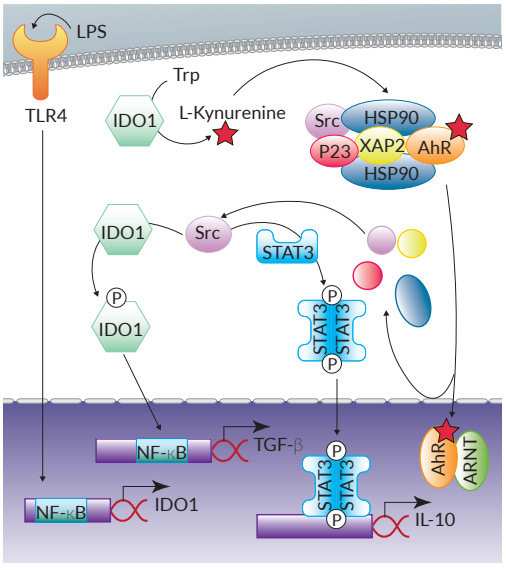
AhR canonical signaling has been extensively reviewed[1]. Briefly, in the absence of ligands crossing the cell membrane, AhR resides in the cytoplasm within a Hsp90:XAP2:p23:Src chaperone protein complex. Upon ligand binding, the complex undergoes conformational changes and translocates into the nucleus. The chaperones are released and AhR heterodimerizes with AhR nuclear translocator (ARNT). The AhR:ligand:ARNT trimer binds to dioxin response elements (DREs) in the upstream regulatory regions of AhR target genes, which include the cytochrome P450-dependent monooxygenase Cyp1a1, the AhR repressor (AhRR), and the IL-22 interleukin. Of note, non-canonical AhR signaling pathways have also been reported, either at the genomic level through association with other transcription factors (e.g. NF-κB), or at the non-genomic level (e.g. through the release of the Src kinase)[2,3].



Besides xenobiotics, including the prototypic AhR agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a variety of dietary-derived AhR ligands have been identified, many of which are byproducts of tryptophan (Trp) amino acid metabolism[4]. AhR has emerged as a regulator of host-microbiome symbiosis. On the one hand, AhR activation by dietary ligands shapes the intestinal bacterial composition[5]. On the other hand, AhR sensing regulates homeostasis and functionality of the gut immune cells[6].

Trp metabolism by the gut microbiota generates AhR agonists which support the development and maintenance of intestinal type 3 innate lymphoid cells (ILC3), the innate counterpart of the adaptive CD4 T cells producing IL-17 and IL-22 (Th17/22). AhR-signaling is also needed for the maintenance of IL-22 producing intraepithelial lymphocytes (IELs). IL-22 is involved in mucosal wound-healing and the production of anti-microbial peptides (AMPs) by intestinal epithelial cells (IECs)[6]. The AhR-IL-22 axis in the gut plays a significant role in the host defense against microbial pathogens, while simultaneously ensuring disease tolerance to limit harmful impact. To this end, there is increasing evidence that the strength of AhR activation modulates CD4 T cell responses. Weak AhR activation supports a pro-inflammatory response (Th17/22), while strong AhR activation promotes induction of tolerogenic DCs and regulatory T cells (Tregs)[6-8].

Different sets of data may provide mechanistic explanations as to how AhR is involved in the regulation of both pro-inflammatory and tolerance responses. While canonical AhR signaling in intestinal immune cells leads to IL-22 production, non-genomic AhR signaling is responsible for at least two suppressive outcomes. Overreacting responses to LPS challenge in mice are repressed through AhR-associated Src kinase phosphorylation of the indoleamine 2,3-dioxygenase IDO1, which in turn induces NF-κB-mediated transcription of the suppressive cytokine TGF-β (transforming growth factor) in DCs[9]. Moreover, Trp degradation by IDO1 provides AhR agonists, such as L-Kynurenine, implicated in Treg generation[9]. Another LPS-induced suppressive effect is the production of the anti-inflammatory cytokine IL-10 via AhR-associated Src kinase and STAT3 signaling in macrophages. Of note, LPS recognition by TLR4 promotes AhR expression in macrophages, but it is unclear whether it is also the case upon stimulation with other pattern recognition receptor ligands[10].



A series of diseases are associated with gut microbiota-immune cells dysbiosis, and whether imbalance is a cause or a consequence remains unclear. In inflammatory bowel disease, immune cells tend to express low levels and impaired activity of AhR, a status maintained by decreased concentration of gut microbiota-derived AhR ligands[2,11]. Colorectal cancer patients display alterations in gut microbiota, increased expression of AhR, chronic IDO1

activation, and reduced Trp concentration in the tumor micro-environment, supporting immune response suppression[11]. Contrarily, spondyloarthritis patients with intestinal symptoms have low gut microbial diversity and Trp catabolism, consistent with inflammation[11]. Also, decreased concentration of circulating microbial catabolites from dietary Trp is implicated in pathogenesis of multiple sclerosis[3]. Hence, AhR plays a key role in gut-microbiota and host's homeostasis, not only in the intestine but also at distant sites. Modulation of the AhR pathways represents an attractive therapeutic strategy. Still, optimization and application of AhR-targeted drugs require a deeper understanding of AhR molecular signaling in different cellular environments and upon varying doses/sources of ligands.

1. Stockinger B. *et al.*, 2014. The aryl hydrocarbon receptor: multitasking in the immune system. Annu. Rev. Immunol. 32:403-32. 2. Lamas B. *et al.*, 2018. Aryl hydrocarbon receptor and intestinal immunity. Mucosal Immunol. 11(4):1024-38. 3. Gutiérrez-Vasquez C. *et al.*, 2018. Regulation of the immune response by the aryl hydrocarbon receptor. Immunity. 48:19-33. 4. Hubbard T.D. *et al.*, 2015. Indole and tryptophan metabolism: endogenous and dietary routes to Ah receptor activation. Drug Metab. Dispos. 43:1522-35. 5. Murray I.A. *et al.* 2016. Expression of the aryl hydrocarbon receptor contributes to the establishment of intestinal microbial community structure in mice. Sci. Rep. 6:33969. 6. Cervantes-Barragan L. & Colonna M. 2018. AHR signaling in the development and function of intestinal immune cells and beyond. Semin. Immunopathol. 40(4):371-77. 7. Ehrlich A.K. *et al.* 2018. TCDD, FICZ, and other high affinity AhR ligands dose-dependently determine the fate of CD4+ T cell differentiation. Toxicol. Sci. 161(2):310-20. 8. Boule L.A. *et al.* 2018. Aryl hydrocarbon receptor signaling modulates antiviral immune responses: ligand metabolism rather than chemical source is the stronger predictor of outcome. Sci. Rep. 8:1826. 9. Bessede A. *et al.* 2014. Aryl hydrocarbon receptor control of a disease tolerance defence pathway. Nature. 511:184-90. 10. Zhu J. *et al.* 2018. Aryl hydrocarbon receptor promotes IL-10 expression in inflammatory macrophages through Src-STAT3 signaling pathway. Front. Immunol. 9:2033. 11. Gao J. *et al.* 2018. Impact of the gut microbiota on intestinal immunity mediated by tryptophan metabolism. Front. Cell. Infect. Microbiol. 8:13.

AhR Ligands

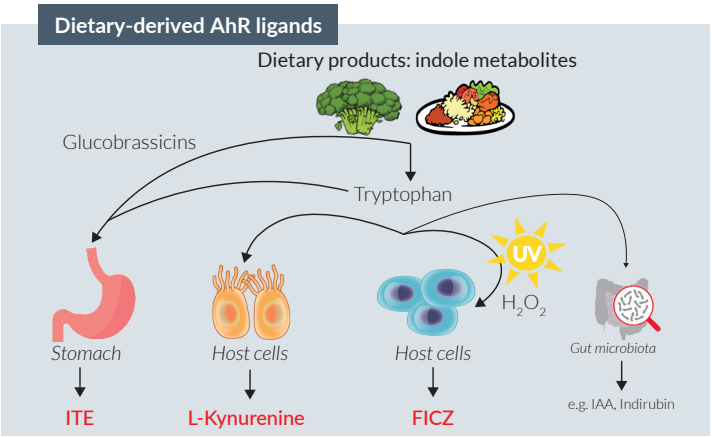
AhR is able to sense a wide range of structurally different exogenous and endogenous molecules. AhR agonists have been found to arise from xenobiotics such as pollutants, and indole metabolites transformed in the stomach and in the gut, as well as in other organs upon photo-oxidation or oxidative stress. InvivoGen provides a selection of dietary-derived AhR ligands as well as a synthetic AhR antagonist to meet your research needs.

- **High quality:** purity > 95%, sterile-filtered, absence of bacterial contamination confirmed
- **Functionally tested:** on HepG2-Lucia™ AhR and HT29-Lucia™ AhR cells (see next page)

AhR Agonists NEW

AhR ligands vary in their structure, and their binding affinity can significantly differ between mouse and human AhR[1]. The prototypic high affinity AhR agonist, the xenobiotic 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), displays a 10-fold higher affinity for mouse AhR compared to human AhR. Conversely, dietary-derived indole metabolites have a better affinity for human AhR; a possible consequence of evolution[1,2].

- **ITE** (2-(1'*H*-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester) is an indole-based AhR ligand that is thought to result from gastric conversion of glucobrassicins (metabolites found in cruciferous vegetables), or from a condensation reaction between two amino acids, tryptophan and cysteine[1,3].
- **L-Kynurenine Plus** is a preparation of L-Kynurenine, a compound generated upon tryptophan metabolism via the kynurenine pathway in host cells (which accounts for more than 90% of tryptophan metabolism)[2,3].
- **FICZ** (6-formylindolol[3,2-*b*]carbazole) is a highly potent AhR ligand that results from tryptophan conversion upon UV-dependent photo-oxidation or H_2O_2-mediated oxidative stress[2,3].



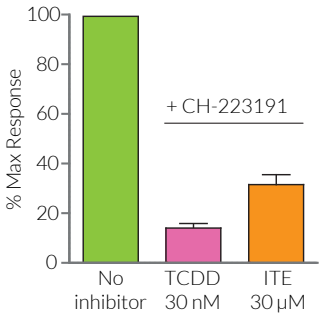
1. Lamas B. *et al.*, 2018. Aryl hydrocarbon receptor and intestinal immunity. Mucosal Immunol. 11(4):1024-38. 2. Murray I.A. *et al.*, 2017. Ligand activation of the Ah receptor contributes to gastrointestinal homeostasis. Curr. Opin. Toxicol. 2:15-23. 3. Hubbard T.D. *et al.*, 2015. Indole and tryptophan metabolism: endogenous and dietary routes to Ah receptor activation. Drug Metab. Dispos. 43:1522-35.

www.invivogen.com/AhR-ligands

AhR Inhibitor

- **CH-223191** - AhR antagonist NEW

CH-223191 (2-methyl-2H-pyrazole-3-carboxylic acid) is a synthetic antagonist of AhR that was first described as a competitive ligand of TCDD[1]. Interestingly, CH-223191 exerts a ligand-selective antagonism and appears to be more effective on halogenated aromatic hydrocarbons such as TCDD than on polycyclic aromatic hydrocarbons and non halogenated aromatic hydrocarbons such as FICZ and ITE, respectively[2].



Inhibiton of AhR activity by CH-223191 in HepG2-Lucia™ AhR cells: Cells were stimulated with 30 nM TCDD or 30 µM ITE in the presence of 10 µM CH-223191. After overnight incubation, the AhR response was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™. Percentages of the maximal response for the ligand with no inhibitor are shown.

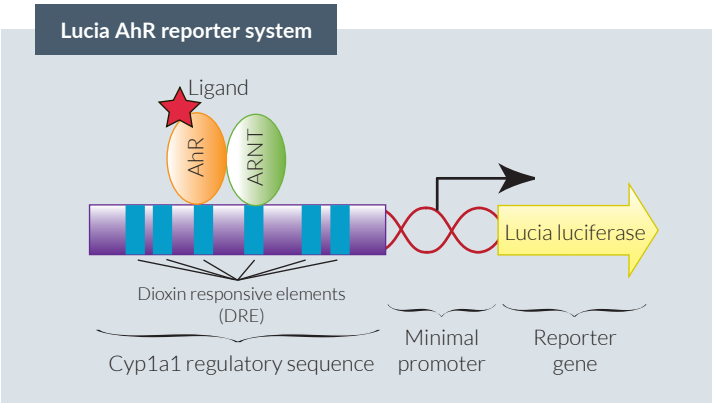
1. Kim, S.H. *et al.*, 2006. Novel compound 2-methyl-2H-pyrazole-3-carboxylic acid (2-methyl-4-o-tolylazo-phenyl)-amide (CH-223191) prevents 2,3,7,8-TCDD-Induced toxicity by antagonizing the aryl hydrocarbon receptor. Mol. Pharmacol. 69:1871-78. 2. Zhao B. *et al.*, 2010. CH223191 is a ligand-selective antagonist of the Ah (Dioxin) receptor. Toxicol. Sci. 117:393-403.

www.invivogen.com/AhR-inhibitors

Aryl Hydrocarbon Receptor Reporter Cells

InvivoGen provides two AhR reporter cell lines engineered from the human HT29 colon adenocarcinoma and HepG2 hepatoma. HT29-Lucia™ AhR cells and HepG2-Lucia™ AhR cells allow the study of AhR activation upon incubation with a wide range of agonists such as xenobiotics and dietary-derived indole products. Importantly, our AhR reporter cells are of human origin and express endogenous human AhR, which makes them highly relevant for screening endogenous AhR agonists in human samples.

- HT29-Lucia™ AhR Cells NEW
- HepG2-Lucia™ AhR Cells NEW



HT29-Lucia™ AhR cells and HepG2-Lucia™ AhR cells report AhR activation through the monitoring of human Cyp1a1-induced Lucia luciferase activity. The microsomal cytochrome P450-dependent mono-oxygenase Cyp1a1 gene is an important AhR target gene. In both cell lines, the Lucia luciferase reporter gene is under the control of the entire regulatory sequence of human Cyp1a1. Upon ligand binding, cytoplasmic AhR undergoes conformational change, transcolates into the nucleus, and associates with ARNT to bind dioxin responsive elements (DREs) in the Cyp1a1 regulatory sequence. AhR activation can be easily assessed by measuring the secreted Lucia luciferase activity in the cell culture supernatant using QUANTI-Luc™. HT29-Lucia™ AhR cells and HepG2-Lucia™ AhR cells are resistant to Zeocin™. Both cell lines have been functionally tested with TCDD, FICZ, ITE, and L-Kynurenine Plus (a preparation of L-Kynurenine).

HT29-Lucia™ AhR Cells

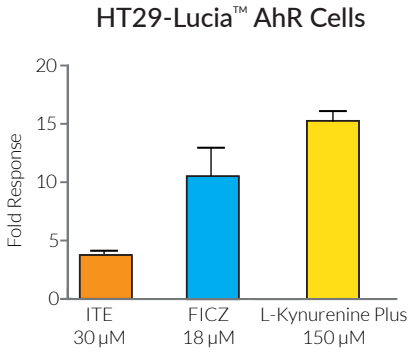
Highly relevant for screening gut microbiota-derived ligands

InvivoGen offers the first AhR reporter cell line derived from the human HT29 colon adenocarcinoma cell line. HT29-Lucia™ AhR cells display multiple advantages. Their anatomical origin and ability to differentiate into intestinal enterocyte-like cells and mucus-producing cells make these cells highly relevant for studying intestinal microbiota-related ligands for AhR. The AhR agonists ITE, FICZ and L-Kynurenine Plus induce significant Lucia luciferase reporter activity at the optimal concentrations of 30 µM, 18 µM and 150 µM, respectively. Importantly, in HT29-Lucia™ AhR cells, Lucia luciferase is not induced by other pattern recognition receptor ligands.

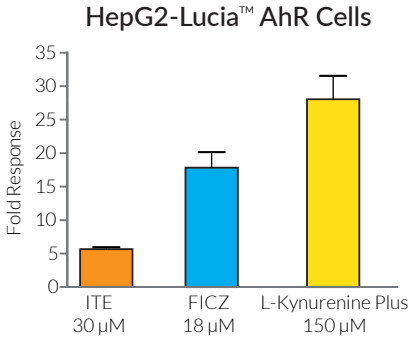
HepG2-Lucia™ AhR Cells

Highly relevant for screening xenobiotic AhR ligands

HepG2-Lucia™ AhR cells derive from the human HepG2 hepatoma cell line which expresses endogenous AhR and is of great interest for the detection/screening of AhR ligands in food or environmental samples. InvivoGen's HepG2-Lucia™ AhR cell-reporter assay allows a sensitive detection of AhR ligands (concentrations as low as 0.02 to 2 µM). HepG2-Lucia™ AhR cells specifically respond to AhR agonists such as ITE, FICZ and L-Kynurenine Plus. Also, in these cells, Lucia luciferase is not induced by other pattern recognition receptor ligands. Of note, InvivoGen's AhR agonists do not induce activation of the interferon regulatory factors nor of the NF-κB transcription factor (as tested on our HepG2-Dual™ cells).



Induction of AhR activity by tryptophan byproducts in HT29-Lucia™ AhR cells: cells were incubated with 100 µM ITE, 18 µM FICZ, or 150 µM L-Kynurenine Plus. After overnight incubation, the AhR activation was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™. Data are expressed as fold responses as compared to non-induced cells.



Induction of AhR activity by tryptophan byproducts in HepG2-Lucia™ AhR cells: cells were incubated with 30 µM ITE, 18 µM FICZ, or 150 µM L-Kynurenine Plus. After overnight incubation, the AhR activation was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™. Data are expressed as fold responses as compared to non-induced cells.

PRODUCT	QUANTITY	CAT. CODE
HepG2-Lucia™ AhR cells NEW	3-7 x 10^6 cells	hpgl-ahr
HT29-Lucia™ AhR cells NEW	3-7 x 10^6 cells	ht2l-ahr
FICZ NEW	1 mg	tlrl-ficz
ITE NEW	10 mg	tlrl-ite
L-Kynurenine Plus NEW	10 mg	tlrl-kynp
CH-223191 NEW	10 mg	inh-ch22
Quanti-Luc™	2 pouches (2 x 25 ml)	rep-qlc1
Quanti-Luc™ Gold	1 pouch (25 ml)	rep-qlcg1
Zeocin™	5 x 1 ml	ant-zn-05

Gut Microbiota-Related Cell Lines

The detection of pathogens by pattern recognition receptors (PRRs) is crucial for initiating the innate immune response. Additionally, PRRs enable microbial colonization of the gut mucosa by many different commensal bacteria, fungi, and viruses. The most well characterized microbial PRRs that recognize these microorganisms are the membrane associated Toll-like receptors (TLRs), C-type lectin receptors (CLRs), and the intracellular nucleotide oligomerization domain (NOD)-like receptors (NLRs)[1,2].

- TLR Reporter Cells
- NOD1/2 Reporter Cells
- Dectin-1a/b Reporter Cells

InvivoGen provides a large collection of both human and murine PRR reporter cell lines and agonists to meet your research needs. The HEK-Blue™ reporter cell lines are a collection of human embryonic kidney (HEK293)-derived cell lines designed for investigating various PRR signaling pathways, by monitoring the activation of NF-κB. HEK-Blue™ cells overexpress the transfected target PRR gene. Additionally, the cells are transfected with a secreted embryonic alkaline phosphatase (SEAP) reporter gene, under the control of a minimal promoter fused to five NF-κB and AP-1-binding sites. Stimulation with a PRR ligand such as di- and triacylated lipoproteins (TLR2/TLR6, TLR2/TLR1, respectively), cytosolic peptidogylcan (NOD1/2), or β-glucans (Dectin-1a/b) activates NF-κB and AP-1, inducing the production of SEAP. The level of SEAP is easily determined using InvivoGen's QUANTI-Blue™ Solution.

PRODUCT		QUANTITY	CAT. CODE
HEK-Blue™ hTLR2		3-7 x10^6 cells	hkb-htlr2
HEK-Blue™ hTLR2-TLR1	NEW	3-7 x10^6 cells	hkb-htlr21
HEK-Blue™ hTLR2-TLR6	NEW	3-7 x10^6 cells	hkb-htlr26
HEK-Blue™ hTLR3		3-7 x10^6 cells	hkb-htlr3
HEK-Blue™ hTLR4		3-7 x10^6 cells	hkb-htlr4
HEK-Blue™ hTLR5		3-7 x10^6 cells	hkb-htlr5
HEK-Blue™ hTLR9		3-7 x10^6 cells	hkb-htlr9
HEK-Blue™ hNOD1		3-7 x10^6 cells	hkb-hnod1
HEK-Blue™ hNOD2		3-7 x10^6 cells	hkb-hnod2
HEK-Blue™ hDectin-1a		3-7 x10^6 cells	hkb-hdect1a
HEK-Blue™ hDectin-1b		3-7 x10^6 cells	hkb-hdect1b

Please see our website for full lists of human and murine PRR reporter cell lines and their agonists.

1. Swiatczak, B. & Cohen, I.R. 2015. Gut feelings of safety: tolerance to the microbiota mediated by innate immune receptors. Microbiol Immunol. 59:573-585. 2. Mu, C. *et al.* 2015. Crosstalk between the immune receptors and gut microbiota. Curr Protein Pept Sci. 16:622-631.

www.invivogen.com/reporter-cells

QUANTI-Blue™ Solution NEW

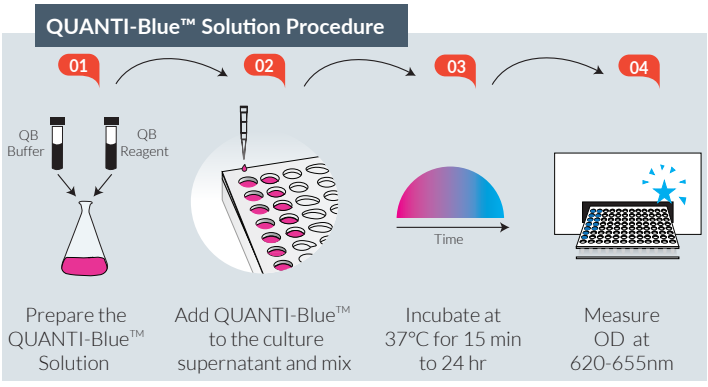
Liquid formulation for SEAP detection

- Easy to prepare: Simply mix the 2 reagents provided
- Convenient: Can be added directly to the cell culture
- Adaptable: For use in high-throughput screening (HTS)

InvivoGen's new 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.

Importantly, QUANTI-Blue™ Solution produces the same results as the powder formulation and offers more convenience. 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.

The QUANTI-Blue™ Solution can be used with InvivoGen's large collection of SEAP reporter cell lines.



PRODUCT		QUANTITY	CAT. CODE
QUANTI-Blue™ Solution	NEW	5 ml	rep-qbs
QUANTI-Blue™ Solution	NEW	Bulk	Please enquire

www.invivogen.com/quanti-blue

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