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. 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.

AhR canonical signaling has been extensively reviewed. Briefly, in the absence of ligands crossing the cell membrane, AhR resides in the cytoplasm within a Hsp90-XAP2: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).

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). 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).

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. Moreover, Trp degradation by IDO1 provides AhR agonists, such as L-Kynurenine, implicated in Treg generation. 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.

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. Colorectal cancer patients display alterations in gut microbiota, increased expression of AhR, chronic IDO1...
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.

AhR Agonists

AhR ligands vary in their structure, and their binding affinity can significantly differ between mouse and human AhR. 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.

- **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.

- **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).

- **FICZ** (6-formylindolol[3,2-b]carbazole) is a highly potent AhR ligand that results from tryptophan conversion upon UV-dependent photo-oxidation or H₂O₂-mediated oxidative stress.

AhR Inhibitor

- **CH-223191 - AhR antagonist**

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. 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.

**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)

Dietary-derived AhR ligands

- Glucobrassicins
- Tryptophan
- ITE
- L-Kynurenine
- FICZ

Dietary products: indole metabolites

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**
- **HepG2-Lucia™ AhR Cells**

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, translocates 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).
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 peptidoglycan (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.

**QUANTI-Blue™ Solution**

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.

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>QUANTITY</th>
<th>CAT. CODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>QUANTI-Blue™ Solution</td>
<td>NEW</td>
<td>rep-qbs</td>
</tr>
<tr>
<td>QUANTI-Blue™ Solution</td>
<td>NEW</td>
<td>Bulk</td>
</tr>
</tbody>
</table>

**QUANTI-Blue™ Solution Procedure**

1. Prepare the QUANTI-Blue™ Solution
2. Add QUANTI-Blue™ to the culture supernatant and mix
3. Incubate at 37°C for 15 min to 24 hr
4. Measure OD at 620-655nm

www.invivogen.com/reporter-cells

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