

Indirubin

AhR agonist

Catalog Code: tlrl-indb

<https://www.invivogen.com/indirubin>

For research use only

Version 19E07-NJ

PRODUCT INFORMATION

Contents

- 10 mg Indirubin

Storage and stability

- Indirubin is provided as a powder and shipped at room temperature. Upon receipt, store product at -20°C.
- Upon resuspension, prepare aliquots and store at -20°C. Resuspended product is stable for at least 1 month when properly stored at -20°C. Avoid repeated freeze-thaw cycles.
- We recommend to protect this product from light.

Quality control

- Purity >95% (HPLC)
- Absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ hTLR2 and HEK-Blue™ hTLR4 cells.
- Biological activity has been validated using cellular assays.

PRODUCT DESCRIPTION

Indirubin (2-(2-oxo-1H-indol-3-ylidene)-1H-indol-3-one) is a dietary-derived indole-based ligand of the cytosolic aryl hydrocarbon receptor (AhR)¹. Indirubin is generated from tryptophan (Trp) metabolism by intestinal bacteria which results in the production of indole. Indole is then further metabolized by the host's hepatocytes into indirubin and other indole-derivatives. Therefore, indirubin is an endogenous AhR agonist, but requires diet-derived Trp, and the activity of commensal gut microbiota¹. Despite indirubin being identified in human urine, it has not yet been established whether the quantities that are synthesized by the liver are sufficient to activate AhR *in vivo*². Using *in vitro* yeast reporter assays, indirubin was shown to be more potent at activating human AhR than 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the prototypical AhR agonist². Of note, indirubin is a more potent agonist of human AhR than murine AhR³.

1. Hubbard T.D. *et al.*, 2015. Indole and tryptophan metabolism: endogenous and dietary routes to Ah receptor activation. *Drug Metab. Dispos.* 43:1522-35.
2. Adachi J. *et al.*, 2001. Indirubin and indigo are potent aryl hydrocarbon receptor ligands present in human urine. *J. Biol. Chem.* 276:31475-78.
3. Flaveny C.A. *et al.*, 2009. Ligand selectivity and gene regulation by the human aryl hydrocarbon receptor in transgenic mice. *Mol. Pharmacol.* 75:1412-20.

CHEMICAL PROPERTIES

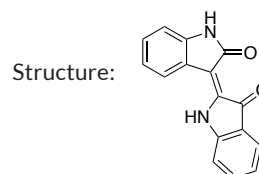
CAS Number: 479-41-4

Synonym: 2-(2-oxo-1H-indol-3-ylidene)-1H-indol-3-one

Formula: C₁₆H₁₀N₂O₂

Molecular weight: 262.26 g/mol

Solubility: 10 mg/ml in DMSO



METHODS

Preparation of 5mM stock solution

1. Add 7.7 ml of DMSO to 10 mg of indirubin. Mix by vortexing.
2. Use immediately or store aliquots at -20°C.
3. Prepare future dilutions using DMEM or EMEM

Working concentration range: 25 - 100 µM for cell culture assays

AhR activation assay

Described below is a protocol to study AhR activation in **HepG2-Lucia™ AhR cells** which derive from the human HepG2 hepatoma cell line. These cells report AhR activation through the monitoring of human Cyp1a1-induced Lucia luciferase activity.

For more information, visit <https://www.invivogen.com/hepg2-lucia-ahr>.

1. Add 20 µl of indirubin at 100 µM (final concentration) per well of a flat-bottom 96-well plate.

Note: we recommend to include a control dilution of DMSO in your assay.

2. Add 180 µl of cell suspension (~20,000 cells) per well.
3. Incubate the plate at 37°C in a 5% CO₂ incubator for 18-24 hours.
4. Monitor Lucia luciferase reporter protein production using a luciferase detection reagent, such as **QUANTI-Luc™**.

RELATED PRODUCTS

Product	Description	Cat. Code
CH-223191	AhR inhibitor	inh-ch22
FICZ	AhR ligand	tlrl-ficz
HepG2-Lucia™ AhR Cells	AhR hepatocytic reporter cells	hpgl-ahr
HT29-Lucia™ AhR Cells	AhR colon cancer reporter cells	ht2l-ahr
ITE	AhR ligand	tlrl-ite
L-Kynurenine	AhR ligand	tlrl-kyn
QUANTI-Luc™	Lucia detection medium	rep-qlc1

TECHNICAL SUPPORT

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