PRODUCT INFORMATION

Contents
L-Kynurenine is available in two pack sizes:
• trl-kyn: 1 x 10 mg  • trl-kyn-5: 5 x 10 mg

Storage and stability
- L-Kynurenine is shipped at room temperature. Store at 15-25 °C.
- Upon resuspension, store at 15-25 °C. Resuspended product is stable for 6 months when properly stored. DO NOT FREEZE.
- We recommend to protect this product from light.

Quality control
- Purity ≥ 95% (UHPLC)
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™TLR2 and HEK-Blue™TLR4 cells.
- The biological activity has been validated using cellular assays.

BACKGROUND

L-Kynurenine (β-Anthraniloyl-L-Alanine) is an endogenous agonist for the cytosolic aryl hydrocarbon receptor (AhR)1. L-Kynurenine results from the catabolic conversion of tryptophan (Trp) by two enzymes, the indoleamine-2,3-dioxygenase (IDO1) and the tryptophan-2,3-dioxygenase (TDO2). L-Kynurenine is the first byproduct of Trp metabolism generated via the enzymatic “kynurenine pathway”, which produces other kynurenine derivatives2. L-Kynurenine is implicated in the production of TGF-β and expansion of regulatory T cells through the AhR-Src-IDO1 pathway. An increase in the kynurenine/Trp ratio in tumors has been shown to correlate with cancer progression, corroborating immuno-suppressive functions for this AhR agonist2. Yet, L-Kynurenine’s mode of action on AhR is still unclear. Indeed, L-Kynurenine could be a low-affinity AhR pro-ligand that is slowly converted in high-affinity compounds acting as AhR agonists at subnanomolar concentrations. This conversion occurs independently of enzymes, when fresh crystalline L-Kynurenine is resuspended as a solution, and gives rise to trace-amounts of derivatives named TEACOPs (trace-extended aromatic condensation products)3.


PRODUCT DESCRIPTION

As stated above, L-Kynurenine may be the pro-ligand for AhR that is converted into trace-amounts derivatives with high-affinity for AhR. To avoid weighing and ensuring the most accurate manipulation of this product, L-Kynurenine is available as 10 mg units. Each lot of L-Kynurenine is functionally tested in cellular assays using our HepG2-Lucia™ AhR and HT29-Lucia™ AhR reporter cells.

CHEMICAL PROPERTIES

CAS number: 2922-83-0
Synonyms: β-Anthraniloyl-L-Alanine, L-2-Amino-4-(2-aminophenyl)-4-oxobutanoic acid
Solubility: 20 mg/ml (96 mM) in DMSO
Formula: C10H12N2O3
Molecular weight: 208.22 g/mol

METHODS

Preparation of 5 mg/ml (24 mM) stock solution
1. Add 1 ml of DMSO to 10 mg of L-Kynurenine. Mix by vortexing and transfer into a new tube.
2. Rinse the commercial vial with 1 ml of DMSO and add to the previously resuspended product. Mix by vortexing. The final volume in the new tube is 2 ml.
3. Use immediately or store at 15-25 °C until required. DO NOT FREEZE.

Working concentration range: 10 μg/ml (48 μM) to 100 μg/ml (480 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. Prepare a 5-fold dilution of L-Kynurenine stock solution in DMSO to obtain a 1 mg/ml work solution.
2. Prepare further dilutions using sterile endotoxin-free water.
3. Add 20 μl of L-Kynurenine at 33 μg/ml (final concentration) per well of a flat-bottom 96-well plate.

Note: we recommend to include a control dilution of DMSO in your assay.
4. Add 180 μl of cell suspension (~20,000 cells) per well.
5. Incubate the plate at 37 °C in a 5% CO2 incubator for 18-24 hours.
6. Monitor Lucia luciferase reporter protein production using a luciferase detection reagent, such as QUANTI-Luc™.

RELATED PRODUCTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Cat. Code</th>
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<td>AhR inhibitor</td>
<td>inh-ch22</td>
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<td>FICZ</td>
<td>AhR ligand</td>
<td>trl-ficz</td>
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<td>HepG2-Lucia™ AhR Cells</td>
<td>AhR hepatoma reporter cells</td>
<td>hpgl-ahr</td>
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<td>HT29-Lucia™ AhR Cells</td>
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<td>QUANTI-Luc™</td>
<td>Lucia detection medium</td>
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