Description

Aryl Hydrocarbon Receptor (AhR) Luciferase Reporter HepG2 Cell Line is a HepG2 cell line designed to monitor the activity of aryl hydrocarbon receptor (AhR). It contains a firefly luciferase reporter driven by multiple copies of the Xenobiotic Response Element (XRE) located upstream of the minimal TATA promoter. Upon ligand binding-induced activation, the receptor translocates to the nucleus, heterodimerizes with ARNT (AhR nuclear translocator), and initiates transcription of the luciferase reporter. This results in a quantifiable luminescent signal that directly correlates with AhR activation.

The cell line has been functionally validated with known AhR agonists FICZ, Tapinarof, MeBio, and the antagonist GNF351 in the presence of MeBio.

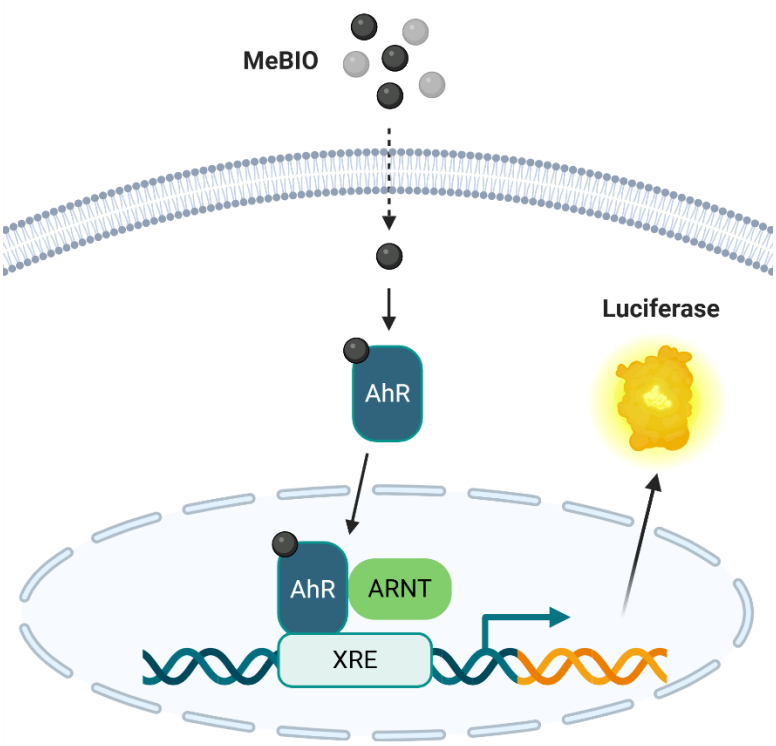

Figure 1: Mechanism of action of Aryl Hydrocarbon Receptor (AhR) Luciferase Reporter HepG2 Cell Line.

Background

Aryl hydrocarbon receptor (AhR) is a ligand-dependent nuclear receptor that functions as a transcription factor. It can follow a canonical signaling pathway where binding of ligands to the cytosolic AhR induces the formation of a complex that translocates to the nucleus where it dimerizes with ARNT (AhR translocator) and binds to XRE (xenobiotic response elements). In addition, it functions in non-canonical pathways involved in immune responses, inflammation, and pathology. Non-canonical signaling pathways involve competition for ARNT, binding to other partners, proteosomal degradation, and/or activation of pathways when in the cytosol, and synergy with other regulatory gene sequences. AhR plays critical roles, functioning as a sensor to the environment, and can respond to POPs (persistent organic pollutants) and other stressors. Its activation can result in the accumulation of toxic metabolic intermediates and liver toxicity, developmental issues, neurotoxicity, amongst others. It also plays a role in cancer, acting as suppressor or promoter in a context-dependent mode. Its contribution to immunoregulation has made it an attractive therapeutic target. Antagonists are being investigated in the cancer therapy field, while agonists play prove useful in inflammatory disorders.

Application

- Validation of AhR agonists and antagonists.
- Mechanistic studies of AhR signaling pathways.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Host Cell

HepG2 cells are an immortalized, adherent, epithelial-like human liver cancer cell line (hepatocellular carcinoma) derived from a 15-year-old male.

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1N	BPS Bioscience #79801

Materials Required for Cellular Assays

Name	Ordering Information
FICZ	BPS Bioscience #84088
Tapinarof	BPS Bioscience #84089
MeBio	BPS Bioscience #84090
GNF351	BPS Bioscience #84091
96-well tissue culture-treated white clear-bottom assay plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells will arrive in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact Technical Support if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. To formulate a comparable but not BPS Bioscience's validated media, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.
Cells should be grown at 37℃ with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1N (BPS Bioscience #79801):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin and 0.5 µg/ml of Puromycin.

Media Required for Functional Cellular Assay

Assay Medium:
Thaw Medium 1 (BPS Bioscience #60187)

Cell Culture Protocol
Note: HepG2 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1.

3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell viability and attachment. For a T25 flask, add 3-4 ml of Thaw Medium 1, and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they reach full confluency. Switch to Growth Medium 1N at first and subsequent passages.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1N and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1N.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5-1:10 once or twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+} and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1N and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze down at least 10 vials of cells at an early passage for future use.

Validation Data

A. Response of Aryl Hydrocarbon Receptor (AhR) Luciferase Reporter HepG2 Cell Line to Agonists

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The assay should include “Stimulated”, “Background Control” and “Unstimulated Control” conditions.

1. Seed AhR Luciferase Reporter HepG2 cells at a density of 20,000 cells per well into a white, clear-bottom 96-well culture plate in 50 µl of Thaw Medium 1. Leave a few empty wells as cell-free control wells (“Background Control”).

2. Incubate cells at 37°C with 5% CO_2 overnight.

3. Prepare a serial dilution of agonist in Thaw Medium 1 at concentrations two times higher than the final concentrations (50 µl/well).

4. Add 50 of each dilution to the wells labeled as “Stimulated”.

5. Add 50 µl of Thaw medium 1 to the “Unstimulated Control” wells (for measuring uninduced level of AhR reporter activity).

6. Add 100 µl of Thaw Medium 1 to “Background Control” wells.

7. Incubate at 37°C with 5% CO_2 overnight (16-22 hours).

8. The expression of luciferase can be measured using the ONE-Step™ Luciferase Assay System (#60690).

9. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of AhR luciferase reporter expression is the background-subtracted luminescence of stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ Stimulated\ Wells - avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells - avg.background}$$

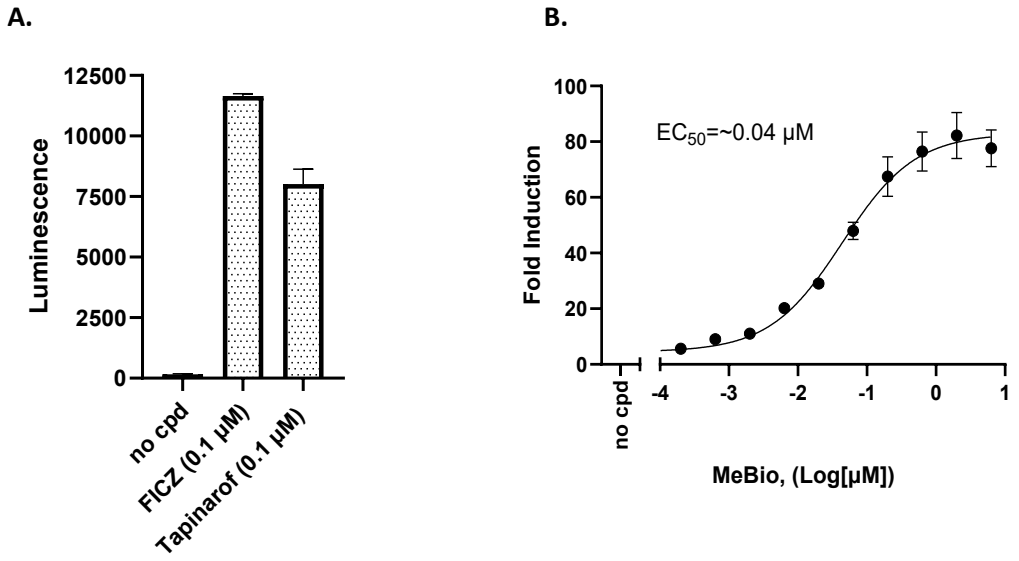

Figure 2: AhR luciferase reporter activity is stimulated by AhR agonists in Aryl Hydrocarbon Receptor (AhR) Luciferase Reporter HepG2 Cell Line.
AhR Luciferase Reporter HepG2 cells were treated with AhR agonists for 18 hours and luciferase activity was measured using the ONE-Step™ Luciferase Assay System. **A.** Bar graph for single dose of FICZ and Tapinarof or **B.** Dose response to MeBio.

B. Response of AhR Luciferase Reporter HepG2 Cell Line to Antagonist

• The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
• All conditions should be performed in triplicate.
• The assay should include "Stimulated, No Antagonist", "Unstimulated, No Antagonist", "Cell-Free Control", and "Stimulated, Antagonist" conditions.

1. Seed AhR Luciferase HepG2 cells at a density of 20,000 cells per well into a white, clear-bottom 96-well culture plate in 50 µl of Thaw Medium 1. Leave a few empty wells as "Cell-Free Control" wells (as background control).

2. Incubate cells at 37°C with 5% CO_2 overnight.

3. Prepare a serial dilution of antagonist in Thaw Medium 1 at concentrations four times higher than the desired final concentrations (25 µl/well).
4. Add 25 of each dilution to the wells labeled as "Stimulated, Antagonist".
5. Add 25 µl of Thaw Medium 1 to the "Stimulated, No Antagonist" and "Unstimulated, No Antagonist" wells.
6. Incubate the cells at 37°C with 5% CO_2 for 30 minutes.
7. Prepare a solution of the agonist MeBio in Thaw Medium 1 at a concentration of 0.4 µM (the final concentration will be 0.1 µM) (25 µl/well).
8. Add 25 µl of MeBio to the "Stimulated, Antagonist" and "Stimulated, No Antagonist" wells.
9. Add 25 µl of Thaw Medium 1 to the "Unstimulated, No Antagonist" (for determining AhR basal activity).
10. Add 100 µl of Thaw Medium 1 to "Cell-Free Control" wells.
11. Incubate the cells at 37°C with 5% CO_2 overnight.
12. Add 100 µl/well of ONE-Step™ Luciferase Assay reagent.
13. Incubate with gentle agitation at room temperature for ~15 to 30 minutes.
14. Measure luminescence using a luminometer
15. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The percent luminescence is the average cell-free control-subtracted luminescence of treated wells divided by the average cell-free control-subtracted luminescence of the "Positive Control" wells (MeBio stimulated, no antagonist antibody) multiplied by 100

$$\%\ Luminescence = \frac{Luminescence\ of\ Treated\ Wells - avg.background}{Avg.\ Luminescence\ of\ Stimulated\ Wells - avg.background} x100$$

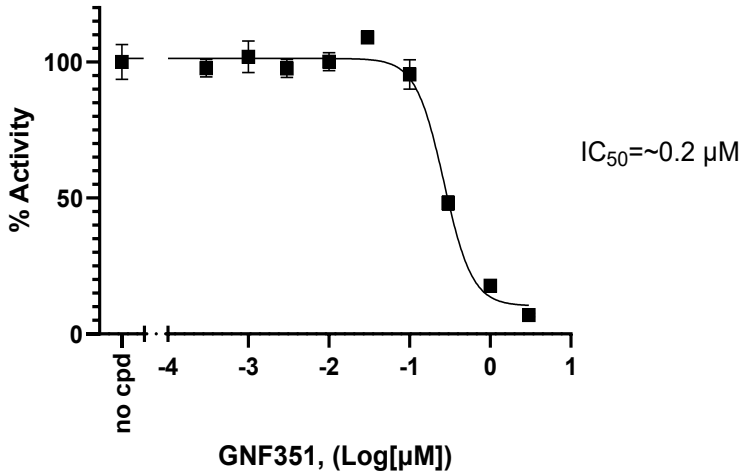

Figure 3: Inhibition of AhR Luciferase Reporter activity by GNF351 in the presence of MeBio stimulation.
AhR Luciferase reporter HepG2 cells were pretreated with increasing concentrations of the AhR antagonist GNF351 for 30 minutes, followed by stimulation with 0.1 µM of MeBio overnight. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System.

Data shown is representative.

References
Coumoul X., *et al.*, 2026 *Signal Transduction and Targeted Therapy* 11: 20.

License Disclosure
Visit bpsbioscience.com/license for the label license and other key information about this product.

Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
XRE Luciferase Reporter Lentivirus	78672	500 µl x2

Version 031026