ARE Luciferase Reporter HepG2 Cell Line (Nrf2 Antioxidant Pathway)

#60513

Description

The antioxidant response element (ARE) Luciferase Reporter HepG2 hepatic cell line contains a stably integrated firefly luciferase gene under the control of ARE, which is recognized by transcription factor Nuclear factor erythroid 2-related factor 2 (Nrf2). Luciferase expression correlates with activation of Nrf2 and the antioxidant pathway. This cell line is validated for its stimulation by tert-butylhydroquinone and sulforaphane.

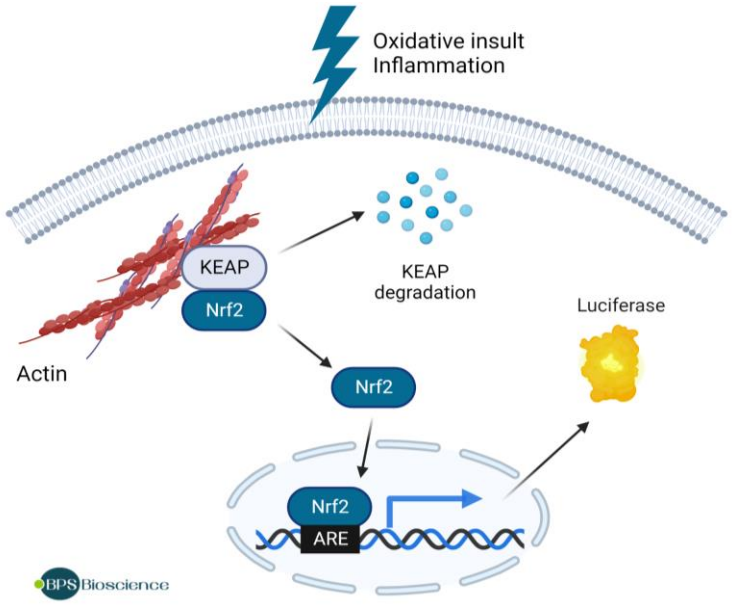

Illustration of the ARE Luciferase Reporter HepG2 Cell Line (Nrf2 Antioxidant Pathway).
Created with BioRender.com

Background

Nrf2 is a transcription factor activated in response to toxic and oxidative drugs and chemicals. Under normal conditions, Nrf2 is retained in the cytosol through its binding to the cytoskeletal protein Kelch-like ECH-associated protein 1 (Keap1). Nrf2 is released from Keap1 upon oxidative stress and translocates to the nucleus, where it binds to the ARE promoter region of genes involved in the oxidative stress response and in drug detoxification, including genes encoding antioxidant enzymes that protect the cell from oxidative damage. Nrf2 supports cellular resistance to carcinogens and to inflammation. Its function is particularly important in the liver, where it contributes to protection against viral hepatitis and alcoholic and nonalcoholic liver disease.

Application

- Monitor Nrf2 antioxidant response pathway.
- Screen for compound activity on the Nrf2 antioxidant response pathway.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of cell freezing medium (BPS Bioscience #79796)

Parental Cell Line

HepG2, Human hepatocellular carcinoma, epithelial-like cells, adherent

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 the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents 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 below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1K	BPS Bioscience #79533

Materials Used in the Cellular Assay

Name	Ordering Information
Tert-butylhydroquinone	Sigma #112941
DL-Sulforaphane	Sigma #S4441
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
White clear-bottom 96-well cell culture plate	
Luminometer	

Storage Conditions

Cells are shipped 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 at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations
For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages.
Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 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 1K (BPS Bioscience #79533):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na-pyruvate, 1% Penicillin/Streptomycin and 600 µg/ml of Geneticin.

Media Required for Functional Cellular Assay

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na-pyruvate, 1% Penicillin/Streptomycin.

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds 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 **(no Geneticin)**.
   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 **(no Geneticin)**.
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 **(no Geneticin)** 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 are fully confluent. At first passage and subsequent passages, use Growth Medium 1K **(contains Geneticin)**.

Cell Passage

These cells grow in clusters rather than spread. The inner cells within the clusters experience contact inhibition of cell division, resulting in a decline of the growth rate. The cells should be split when the clusters appear dense, rather than when the flask surface area is covered. Resuspending the culture to single cells with each split will maximize the growth rate.

1. When much of the surface area of the flask is covered with cells, or clusters appear dense, aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.05% Trypsin/EDTA. Pipet up and down until a single cell suspension is obtained (check under the microscope).
2. Once the cells have detached, add 10 ml of Growth Medium 1K and transfer to a centrifugation tube. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1K **(contains Geneticin)**. Seed into new culture vessels at the desired sub-cultivation ratio of 1:4 to 1:5 twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 1K 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 Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.
4. Dispense 1 ml of cell aliquots into cryogenic vials. 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 storage.

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

A. Activation of ARE reporter activity by inducers of the Nrf2-mediated antioxidant response.

- The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.
- The experiments should be performed in triplicate.

1. Seed ARE Luciferase Reporter HepG2 cells at a density of ~ 40,000 cells in 50 µl/well of Thaw Medium 1 into a white clear-bottom 96-well cell culture plate.
2. Dilute an inducer of the antioxidant response (for example tert-butylhydroquinone or DL-Sulforaphane) at concentrations that are 2-fold higher than the desired final concentrations, in Thaw Medium 1.
    a. Add 50 µl of the dilutions to the cells. The final concentration of DMSO can be up to 0.5%.
    b. Add 50 µl of Thaw Medium 1 containing the same concentration of DMSO as the agonist dilution to "unstimulated control" wells.
    c. Add 100 µl of Thaw Medium 1 containing the same concentration of DMSO as the agonist dilution to "cell-free control" wells (for determining the background luminescence).
3. Incubate the cells at 37°C in a CO_2 incubator for 15 to 18 hours.
4. Perform the luciferase assay using the ONE-Step™ Luciferase Assay System. Add 100 µl of ONE-Step™ Luciferase Assay reagent to all wells and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer.
5. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of luciferase activity is the background-subtracted luminescence of stimulated cells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Lumin.\,stimulated\ cells - ave.\,background}{Lumin.\,unstimulated\ cells - ave.\,background}$$

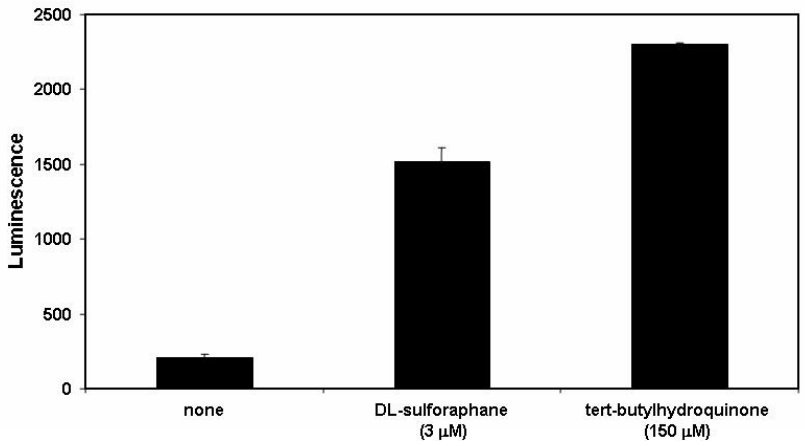

Figure 1: ARE Luciferase Reporter HepG2 cells response to sulforaphane (3 µM) and tert-butylhydroquinone (160 µM).

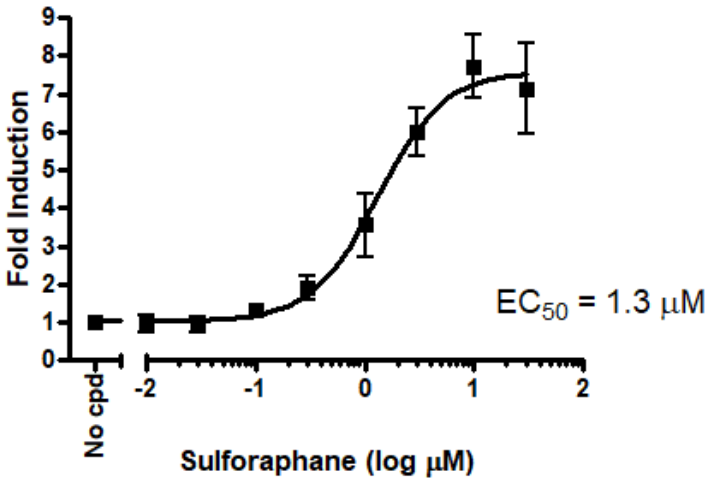

Figure 2: Dose response of sulforaphane in ARE Luciferase Reporter HepG2 cells. The results are shown as fold induction of unstimulated luciferase activity.

References

Cuadrado A, *et al.* Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases (2019) *Nature Rev. Drug Discovery*, **18:** 295-317.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
ARE Reporter Kit (Nrf2 Antioxidant Pathway)	60514	500 reactions
Hedgehog Pathway Gli Luciferase Reporter NIH3T3 Cell Line	60409	2 vials
ARE Luciferase Reporter Lentivirus	79869	500 µl x 2
CRISPRa (SAM) HepG2 Cell Line	78194	2 vials
PARP7 Chemiluminescent Assay Kit	79729	96 and 384 reactions