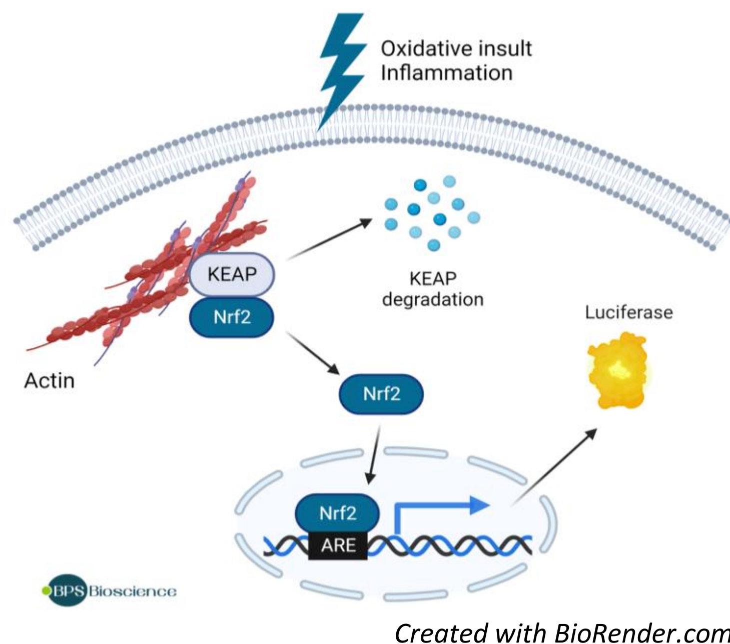
ARE Luciferase Reporter HepG2 Cell Line (Nrf2 Antioxidant Pathway)

Description

The antioxidant response element (ARE) Luciferase Reporter HepG2 hepatic cell line contains a stably integrated firefly luciferase reporter under the control of ARE, which is recognized by the transcription factor Nuclear factor erythroid 2-related factor 2 (Nrf2). Luciferase expression correlates with activation of Nrf2 and the antioxidant pathway. This cell line was validated by stimulation with tert-butylhydroquinone, sulforaphane, and RTA-408 (omaveloxone).



Created with BioRender.com

Figure 1: Illustration of the ARE Luciferase Reporter HepG2 Cell Line (Nrf2 Antioxidant Pathway) mechanism.

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, and dysfunction can lead to neurological diseases such as Alzheimer disease, Parkinson’s and Huntington’s diseases and amyotrophic lateral sclerosis (AMS). Its function is particularly important in the liver, where it contributes to protection against viral hepatitis and alcoholic and nonalcoholic liver disease. Molecules able to interact with Nrf2 and enhance is protective role are of great interest in medicine.

Application

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

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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)

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
RTA-408 (Omaveloxolone)	MedChemExpress #HY-12212
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, the use of validated and optimized media from BPS Bioscience is *highly recommended*. 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 to maintain selective pressure on the cell population expressing the gene of interest.
Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

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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.
   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 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 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.

Cell Passage

These cells grow in clusters rather than fully spread in the dish. 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 most of the surface area of the flask is covered with cells, or clusters appear dense, aspirate the medium and wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}.

2. 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).

3. Once the cells have detached, add 10 ml of Growth Medium 1K and transfer to a centrifugation tube.

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

5. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:4 to 1:5 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 0.05% 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 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 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 conditions should be tested in triplicate.
- The assay should include “Cell-Free Control”, ‘Unstimulated Control” and “Test Condition” wells.

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. Keep a few wells empty to measure background luminescence.

2. Dilute an inducer of the antioxidant response (for example tert-butylhydroquinone, DL-Sulforaphane or RTA-408) at concentrations that are 2-fold higher than the desired final concentrations.

   2.1 For inducers soluble in Thaw Medium 1, prepare a serial dilution in Thaw Medium 1 at 2x the desired concentrations.

   Thaw Medium 1 is the Diluent Solution.

   2.2 For inducers soluble in DMSO prepare a stock solution in 100% DMSO at a concentration 400x higher than the highest desired final concentration, then dilute it 200-fold with Thaw Medium 1 to prepare the highest concentration of the serial dilution. The concentration of DMSO is now 0.5% DMSO.

   Prepare a serial dilution at the desired concentrations using Thaw Medium 1 containing 0.5% DMSO.

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For controls use Thaw Medium 1 with 0.5% DMSO (Diluent Solution).

Note: The concentration of DMSO should not exceed 0.5% in the final reaction.

3. Add 50 µl of the inducer dilutions to the “Test Condition” cells.

4. Add 50 µl of Diluent Solution to the “Unstimulated Control” wells.

5. Add 100 µl of Diluent Solution to the “Cell-Free Control” wells (for determining the background luminescence).

6. Incubate the cells at 37°C in a CO_2 incubator for 15 to 18 hours.

7. Add 100 µl of ONE-Step™ Luciferase Assay reagent to all wells.

8. Rock at room temperature for ~15 minutes.

9. Measure luminescence using a luminometer.

10. 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.

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

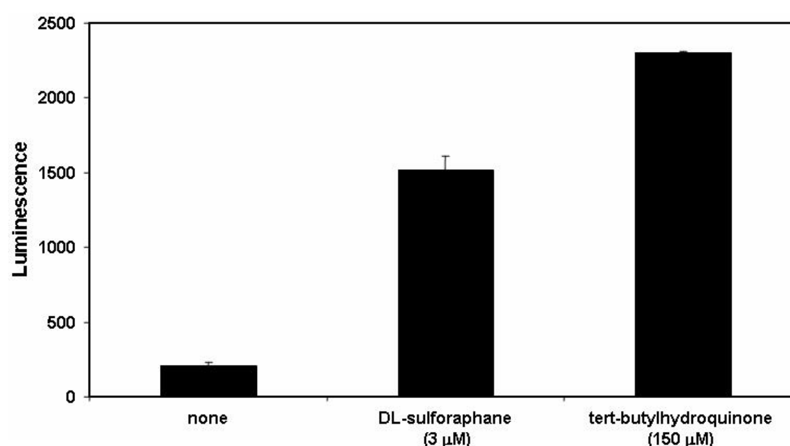

Figure 2: ARE Luciferase Reporter HepG2 cells response to 3 µM sulforaphane and 160 µM tert-butylhydroquinone.
ARE activity was measured with ONE-Step™ Luciferase Assay System.

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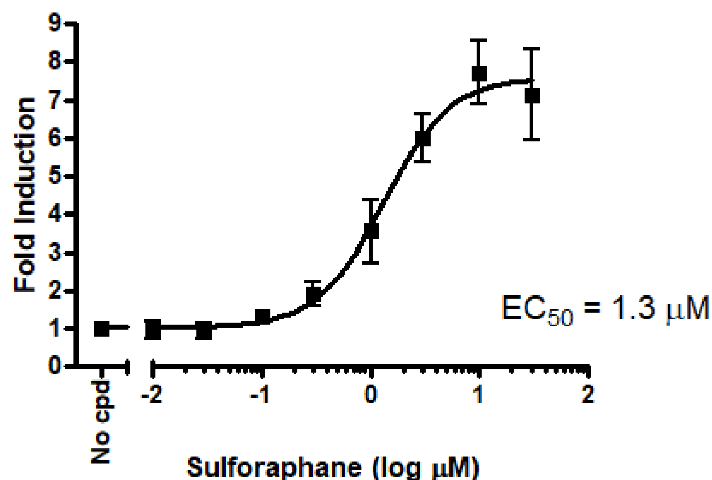

Figure 3: Dose response of ARE Luciferase Reporter HepG2 cells to sulforaphane.
ARE activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction (relative to the "Unstimulated" condition).

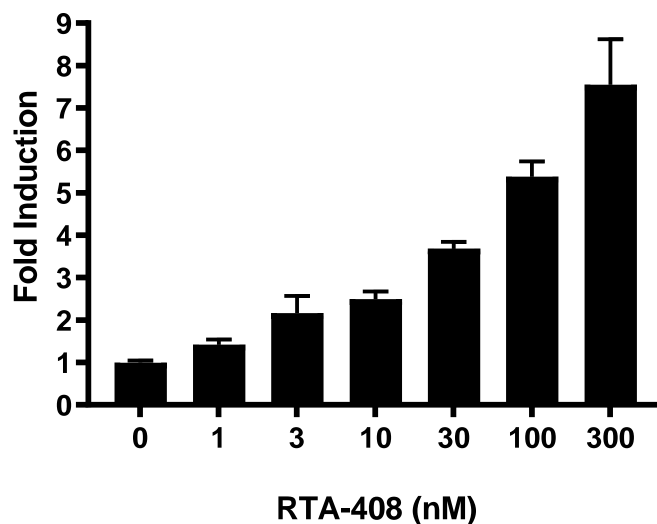

Figure 4: Dose response of ARE Luciferase Reporter HepG2 cells to RTA-408.
ARE activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction (relative to the "Unstimulated" condition).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Cuadrado A, *et al.* 2019 *Nature Rev. Drug Discovery*, 18: 295-317.
Zgorzynska E., *et al.*, 2021 *Int J Mol Sci*, 22(17): 9592.

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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
ARE Luciferase Reporter Lentivirus	79869	500 µl x 2
CRISPRa (SAM) HepG2 Cell Line	78194	2 vials
KEAP1, His-Tag Recombinant	70040	100 µm/1 mg
KEAP1-Nrf2 Inhibitor Screening Assay Kit	72020	96 reactions