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Data Sheet

ARE Reporter – HepG2 Cell line (Nrf2 Antioxidant Pathway)
Catalog #: 60513

Product Description
The Nrf2 antioxidant response pathway plays an important role in cellular antioxidant defense. Nrf2, a basic leucine zipper transcription factor, induces the expression of antioxidant and phase II enzymes by binding to the ARE (antioxidant response element) region of the gene promoter. Under basal conditions, Nrf2 is retained in the cytosol by binding to the cytoskeletal protein Keap1. Upon exposure to oxidative stress or other ARE activators, Nrf2 is released from Keap1 and translocates to the nucleus, where it can bind to the ARE, leading to the expression of antioxidant and phase II enzymes that protect the cell from oxidative damage.

The ARE Reporter – Hep G2 cell line contains a firefly luciferase gene under the control of ARE stably integrated into Hep G2 cells. This cell line is validated for the response to the stimulation of tert-butylhydroquinone and sulforaphane.

Application
- Monitor Nrf2 antioxidant response pathway activity.
- Screen for activators or inhibitors of Nrf2 antioxidant response pathway.

Format
Each vial contains ~2 X 10^6 cells in 1 ml of 10% DMSO.

Storage
Immediately upon receipt, store in liquid nitrogen.

General Culture Conditions

Thaw Medium 1 (BPS Bioscience, #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Life Technologies #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na-pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

Growth Medium 1K (BPS Bioscience, #79533): Thaw Medium 1 (BPS Bioscience, #60187) and 600 μg/ml of Geneticin (Life Technologies #11811031).

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Culture Conditions
Thawing Cells: Prepare a 15mL conical tube with 10 ml of pre-warmed Thaw Medium 1 (no geneticin). Quickly thaw cells in a 37°C water bath with constant and slow agitation. Clean the outside of the vial with 70% ethanol and immediately transfer the entire contents to the conical tube. Spin cells at 100 x g for 5 minutes, and remove all medium from the pellet. Resuspend in 15mL Thaw Medium 1 (no geneticin) and transfer to a T-75 flask. Gently rock the flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO_2. 24 hours after incubation, change culture to fresh Thaw Medium 1 (no geneticin); avoid disturbing the attached cells. Continue to monitor growth for 2-3 days and change the media to remove dead cell debris, if necessary. Begin adding Growth Medium 1K after multiple cell colonies (in clumps) start to appear (indicative of healthy cell division).

Subculture: Cells will grow in clumps rather than spreading throughout the flask. The cells inside the clumps will experience contact inhibition of cell division when the clumps become too dense, and the growth rate will decline. Split the cells when the interior of the clumps appears to be confluent, rather than when the flask surface area is covered. If the cells are split to a single cell at each split (rather than just detaching the clumps from the plate), the growth rate will be maximized.

When cells have reached 90% confluency, remove Growth Medium 1K and gently wash cells twice with PBS (without Magnesium or Calcium). Treat cells with 2 ml of 0.25% tryspin/EDTA and incubate for 2-3 minutes at 37°C. Pipet the trypsinized cells until the clumps have been reduced to single cells under a light microscope. Dispense 10 ml of pre-warmed Growth Medium 1K to trypsinized cells and gently pipette up and down to neutralize trypsin. Transfer cells to a conical tube and centrifuge at 200 x g for 5 minutes. Remove Growth Medium 1K and re-suspend cells in 10 ml of prewarmed Growth Medium 1K. Dispense 2 ml of cell suspension into a new T-75 flask containing prewarmed 15 ml of Growth Medium 1K. Incubate cells in a humidified 37°C incubator with 5% CO2.

Recommended split ratio: 1:4 to 1:5 twice per week.

Cryopreservation: When cells reach 90% confluency, use trypsin to remove cells from plate, spin cells, and remove medium from the pellet. Resuspend the cells in freezing medium (10% DMSO in FBS). Freeze cells using a reduced rate freezing box (-0.5°C to -1°C per minute) down to -80°C, then move cells to liquid nitrogen for long term storage. BPS recommends preparing frozen stocks so cells are not used beyond passage 20.

Mycoplasma Testing
This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Cat. #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Cat. # LT07-518) was used as a positive control.

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Materials Required but Not Supplied

- Tert-butylhydroquinone (Sigma # 112941). Prepare stock solution in DMSO.
- DL-Sulforaphane (Sigma # S4441). Prepare stock solution in DMSO.
- Assay medium: Thaw Medium 1 (BPS Bioscience, #60187)
- Growth Medium 1K (BPS Bioscience, #79533)
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- ONE-Step™ Luciferase Assay System (BPS Bioscience, #60690).
- Luminometer

Mycoplasma testing

The cell line has been screened using the PCR-based VenorGeM® Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of Mycoplasma species.

Functional Validation and Assay Performance

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.

A. Response of ARE Reporter - Hep G2 cells to antioxidant inducers.

1. Harvest ARE Reporter – Hep G2 cells from culture in Growth Medium 1K and seed cells at a density of ~ 40,000 cells per well into white clear-bottom 96-well microplate in 45 µl of assay medium.

2. Dilute antioxidant inducer (tert-butylhydroquinone or DL-Sulforaphane) into assay medium and add 5 µl of dilution to each well. The final DMSO concentration can be up to 0.5%.
   Add 5 µl of assay medium with same concentration of DMSO but without the antioxidant inducer to the unstimulated control wells.
   Add 50 µl of assay medium with DMSO to cell-free control wells (for determining background luminescence).
   Set up each treatment in at least triplicate.

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

4. The next day, perform luciferase assay using the ONE-Step™ Luciferase Assay System: Add 100 µl of ONE-Step™ Luciferase Assay reagent per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer.
   If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.

5. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

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The fold induction of ARE luciferase reporter expression = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells.

Figure 1. ARE Reporter – Hep G2 cell response to antioxidant inducer.

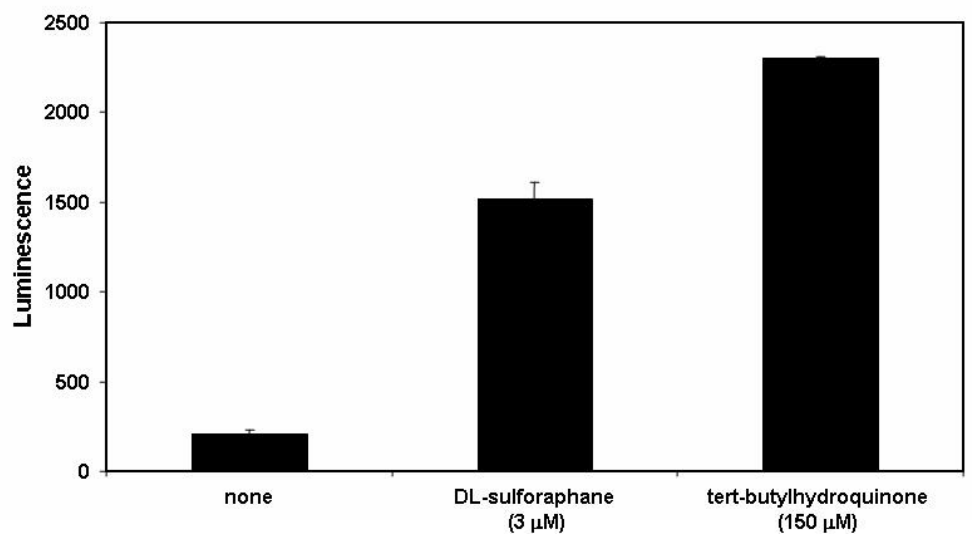
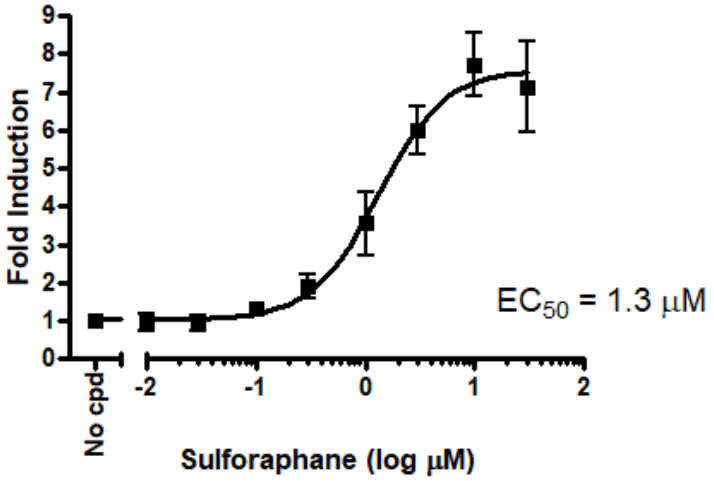


Figure 2. Dose response of ARE Reporter – Hep G2 cells to DL-Sulforaphane. The results are shown as fold induction of ARE luciferase reporter expression.



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References

1. Lee, JM *et. al*. (2004) An important role of Nrf2-ARE pathway in the cellular defense mechanism. *J Biochem Mol Biol.* **37(2):** 139-143.
2. Dinkova-Kostova, AT *et.al.* (2002) Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc Natl Acad Sci U S A.* **99(18):** 11908-11913.

Related Products

Product Name	Catalog #	Size
ARE Reporter Kit (Nrf2 Antioxidant Pathway)	60514	500 rxns
ERK Signaling Pathway SRE Reporter – HEK293 Cell Line	60406	2 vials
Hedgehog Pathway Gli Reporter – NIH3T3 Cell Line	60409	2 vials
JAK/STAT Signaling Pathway ISRE Reporter – HEK293 Cell Line	60510	2 vials
JNK Signaling Pathway AP1 Reporter – HEK293 Cell Line	60405	2 vials
NK-kB Reporter (Luc) – HEK293 Cell Line	60650	2 vials
RARalpha Reporter (Luc) – HEK293 Cell Line	60503	2 vials
Wnt Signaling Pathway TCF/LEF Reporter – HEK293 Cell Line	60501	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
ONE-Step™ Luciferase Assay System	60690-3	1 L

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