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Data Sheet

Nrf2 Antioxidant Pathway
ARE Reporter – Hep G2 Cell line
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 Cat. #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 Cat. #79533): Thaw Medium 1 (BPS Cat. #60187) and 600 μg/ml of Geneticin (Life Technologies #11811031).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1K (Thaw Medium 1 plus Geneticin). It may be necessary to adjust the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium.

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To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 1 **(no geneticin),** spin down cells, and resuspend cells in pre-warmed Thaw Medium 1 **(no geneticin).** Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator. At first passage, switch to Growth Medium 1K **(contains geneticin)**. Cells should be split before they reach complete confluence.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, add Growth Medium 1K and transfer to a tube. Spin down cells, resuspend cells, and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:5 to 1:10 weekly.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA. Add Growth Medium 1K and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS). Place at -80°C overnight and place in liquid nitrogen the next day. Alternatively, vials may be placed directly in liquid nitrogen.

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.

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 Cat. #60187)
- Growth Medium 1K (BPS Cat. #79533)
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- ONE-Step™ Luciferase Assay System (BPS Cat. #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.

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.

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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.
   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.

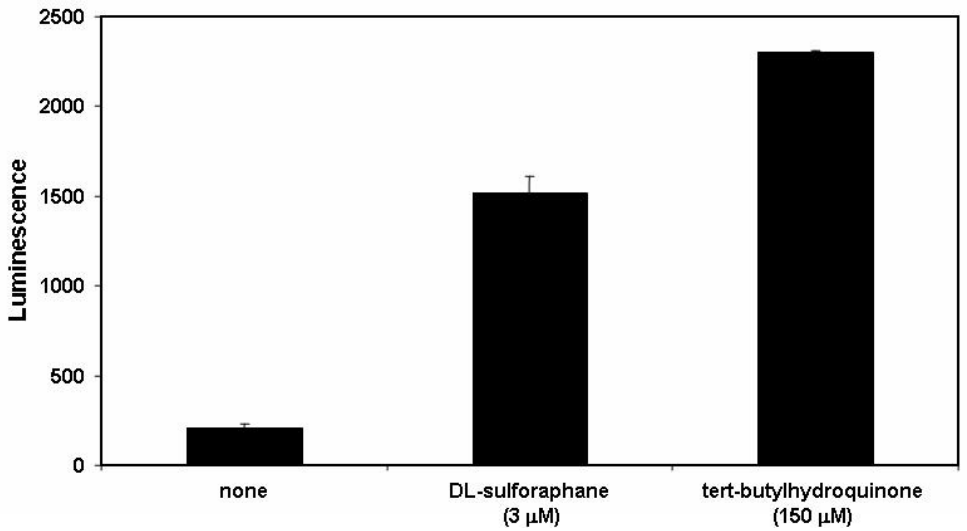

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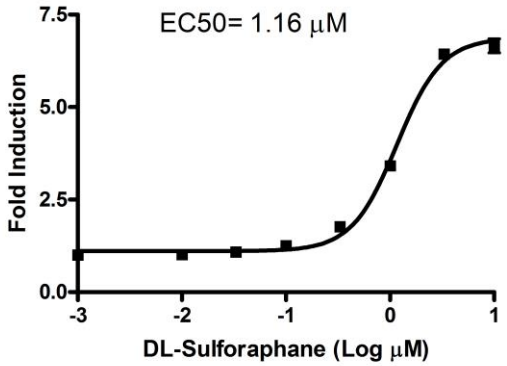
Figure 2. Dose response of ARE Reporter – Hep G2 cells to DL-Sulforaphane. The results are shown as fold induction of ARE luciferase reporter expression.



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
ERK Signaling Pathway SRE Reporter – HEK293 Cell Line	60406	1 vial
Hedgehog Pathway Gli Reporter – NIH3T3 Cell Line	60409	1 vial
JAK/STAT Signaling Pathway ISRE Reporter – HEK293 Cell Line	60510	1 vial
JNK Signaling Pathway AP1 Reporter – HEK293 Cell Line	60405	1 vial
NK-kB Reporter (Luc) – HEK293 Cell Line	60650	1 vial
RARalpha Reporter (Luc) – HEK293 Cell Line	60503	1 vial
Wnt Signaling Pathway TCF/LEF Reporter – HEK293 Cell Line	60501	1 vial
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml

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