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Data Sheet

RARγ Reporter (Luc) - HEK293 Cell Line
Catalog #: 60604

Background
Retinoic acid receptor (RAR) belongs to the family of nuclear receptors and has three subtypes, RARα, RARβ, and RARγ. RAR heterodimerizes with retinoic X receptor (RXR) and acts as a transcription factor that regulates the growth and differentiation of both normal and malignant cells. When RAR binds to its ligands, all-*trans* retinoic acid or 9-*cis* retinoic acid, RAR/RXR heterodimer binds to retinoic acid response elements in the promoter region of target genes and recruits coactivator proteins, leading to transcription and expression of the downstream target genes.

Descriptions
The RAR gamma Reporter (Luc)-HEK293 cell line is designed for monitoring the activity of RARγ. The RAR gamma Reporter (Luc)-HEK293 cell line contains a firefly luciferase gene under the control of retinoic acid response elements stably integrated into HEK293 cells along with full length human RARγ (accession #P13631-1).
This cell line is functionally validated for the response to the stimulation of all-*trans* retinoic acid. The expression of RARγ is confirmed by western blotting.

Application
- Monitor RARγ-regulated pathway activity
- Screen agonists or antagonists of RARγ.

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

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

Storage
Immediately upon receipt, store in liquid nitrogen.

General Culture Conditions

Thaw Medium 6 (BPS Cat. #60183): DMEM medium (Hyclone #SH30243.01) supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

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Complete Growth Medium: Thaw Medium 6 (BPS Cat. #60187) and 400 μg/ml of Geneticin (G418) (Invitrogen #11811031), 1 μg/ml of Puromycin (Hyclone #SV30075.01), and 100 μg/ml Hygromycin (Hyclone #SV30070.01).

Cells should be maintained at 37°C with 5% CO_2 using complete growth medium. If culturing cells in medium from other vendors, it may be necessary to lower the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium.

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 6 **(no Geneticin, Puromycin, and Hygromycin)**, spin down cells, and resuspend cells in pre-warmed Thaw Medium 6 **(no Geneticin, Puromycin, and Hygromycin)**. Transfer resuspended cells to a T25 flask and culture in a 37°C CO_2 incubator. At first passage, switch to complete growth medium **(Thaw Medium 6, Geneticin, Puromycin, and Hygromycin)**. 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 Trypsin/EDTA, and add complete growth medium. Transfer to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:10 to 1:20, twice a week.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA. Add complete growth medium 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 assay in different tissue culture formats, cell number and reagent volume should be scaled appropriately.

Materials Required but Not Supplied

- all-*trans* retinoic acid (ATRA) (Sigma #R2625): make 1 mM stock solution in DMSO
- Assay medium: phenol red-free DMEM + 10% charcoal stripped FBS (Hyclone #SH3006802) + 1% Pen/Strep
- 96-well tissue culture treated white clear-bottom assay plate (Corning #3610)
- ONE-Step™ Luciferase Assay System (BPS, Cat. #60690)
- Luminometer

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Assay protocol: Dose response of RAR gamma Reporter (Luc) - HEK293 cells to all-*trans* retinoic acid (ATRA)

1. One day before plating the cells, remove the growth medium from RAR gamma Reporter (Luc)-HEK293 cells and replace with assay medium for 24 hours.

2. Harvest RAR gamma Reporter (Luc)-HEK293 cells and seed cells in 40 µl of assay medium at a density of ~30,000 cells per well into white clear-bottom 96-well microplate.

3. Prepare threefold serial dilution of ATRA in assay medium and add 10 µl of ATRA solution to each well. The final DMSO concentration is 0.1%.
   Add 10 µl of assay medium with 0.5% DMSO to the unstimulated control wells.
   Add 50 µl of assay medium with 0.1% DMSO to cell-free control wells (for determining background luminescence).
   Set up each treatment in at least triplicate.

4. Incubate cells at 37° in a CO_2 incubator for ~16 to 24 hours.

5. Perform luciferase assay using ONE-Step™ Luciferase Assay System according to the protocol provided: Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~10 minutes. Measure luminescence using a luminometer.
   If using other luciferase reagents from other vendors, follow the manufacturer's assay protocol.

6. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells.
   The fold induction of RAR luciferase reporter expression = background-subtracted luminescence of ATRA-stimulated well / average background-subtracted luminescence of unstimulated control wells

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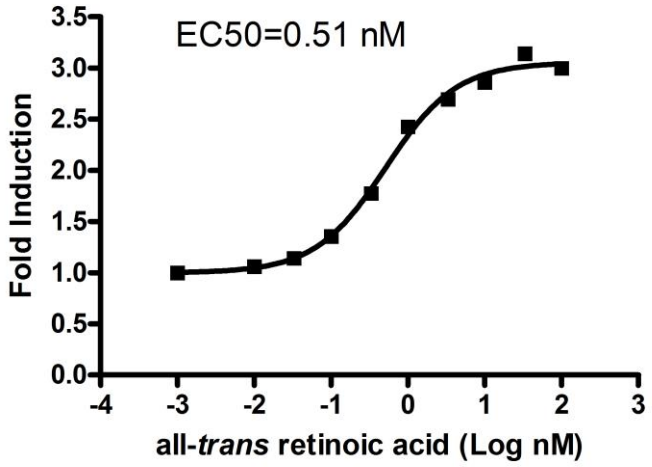
Figure 1. Dose response of RAR gamma Reporter (Luc) - HEK293 cells to all-*trans* retinoic acid (ATRA). Results are shown as fold induction of RAR luciferase reporter expression.



References

1. Petkovich, M, *et al. Nature* (1987) **330(6147)**: 444-450.
2. Allenby, G, *et al*. *Proc. Natl. Acad. Sci. USA* (1993) **90(1):** 30-34.

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