Description

RARβ Luciferase Reporter HEK293 Cell Line is a HEK293 cell line engineered to express firefly luciferase, under the control of retinoic acid response elements, and with full length human RARβ (accession #P10826-2). RARβ Luciferase Reporter HEK293 Cell Line is designed for monitoring the activity of RARβ.
This cell line was functionally validated by stimulation with all-*trans* retinoic acid (ATRA).

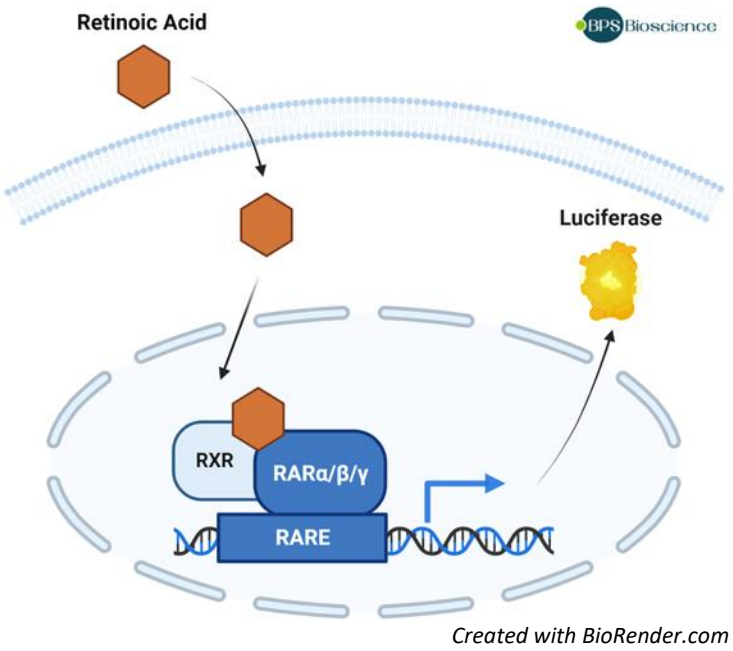

Figure 1: Illustration of the mechanism of action of RARγ Luciferase Reporter HEK293 Cell Line. Upon activation with retinoic acid, RAR dimerizes with RXR, and activates RARE and luciferase expression. The level of activation corresponds directly to the luciferase signal.

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. RARβ dysfunction can result in cervical carcinoma. Abnormal promoter DNA hypermethylation has also been linked to cancer.

Application

- Monitor RARβ-regulated pathway activity.
- Screen for agonists or antagonists of RARβ.

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
HEK293, Human Embryonic Kidney, 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 6	BPS Bioscience #60183
Growth Medium 6A	BPS Bioscience #79542

Materials Required for Cellular Assay

Name	Ordering Information
ATRA	Sigma #R2625
Assay Medium 6A	BPS Bioscience #82211
96-well tissue culture treated white clear-bottom assay plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
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.

Media Required for Cell Culture

Thaw Medium 6 (BPS Bioscience #60183):
DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 6A (BPS Bioscience #79542):
DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 400 µg/ml of Geneticin (G418), 1 µg/ml of Puromycin, and 100 µg/ml Hygromycin B.

Media Required for Functional Cellular Assay

Assay Medium 6A (BPS Bioscience #82211):
Phenol red-free DMEM, 10% Charcoal Stripped FBS, and 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 6.
   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 6.

3. Transfer the resuspended cells to a T25 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 6 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 6A.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (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 6A and transfer to a tube.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 6A.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:10 to 1:20 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 1G 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. Dose response of RARβ Reporter Luciferase HEK293 Cell Line to all-*trans* retinoic acid (ATRA)

- 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.
- The assay should include "Cell-Free Control", "Unstimulated Control" and "Test Condition" wells.

Assay Medium:
Assay Medium 6A.

1. The day prior to initiating the assay exchange the media used to culture RARβ Reporter Luciferase HEK293 cells to Assay Medium.

2. Seed RARβ Reporter Luciferase HEK293 cells at a density of ~30,000 cells in 40 µl/well of Assay Medium into a white clear-bottom 96-well cell culture plate. Leave some wells with only Assay Medium for background determination.

3. Prepare a stock solution of ATRA in 100% DMSO at 1000-fold than the highest desired final concentration, then dilute it 200-fold with Assay Medium 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 Assay Medium containing 0.5% DMSO.

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

   Note: The concentration of DMSO should not exceed 0.1% in the final reaction.

4. Add 10 µl of the ATRA dilutions to the "Test Condition" cells.

5. Add 10 µl of Diluent Solution to the "Unstimulated Control" and "Cell-Free Control" wells (for determining the background luminescence).

6. Incubate the cells at 37°C in a CO_2 incubator for 16 to 24 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.

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

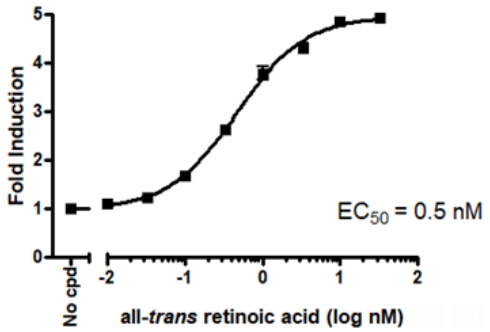

Figure 2: Dose response curve of RARß Luciferase Reporter HEK293 Cell Line to all-trans retinoic acid (ATRA). Cells were incubated with increasing amounts of ATRA. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. Results are shown as fold induction of RAR luciferase reporter expression.

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

References

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

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

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 reactions/384 reactions

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