Description

RARα Luciferase Reporter HEK293 Cell Line are engineered HEK293 cells expressing a conditional firefly luciferase reporter under the control of retinoid acid response elements (RARE), and constitutively expressing full length human Retinoic Acid Receptor alpha (RARα, NM_0.00964). This cell line is functionally validated to respond to *all-trans* retinoic acid (ATRA) stimulation.

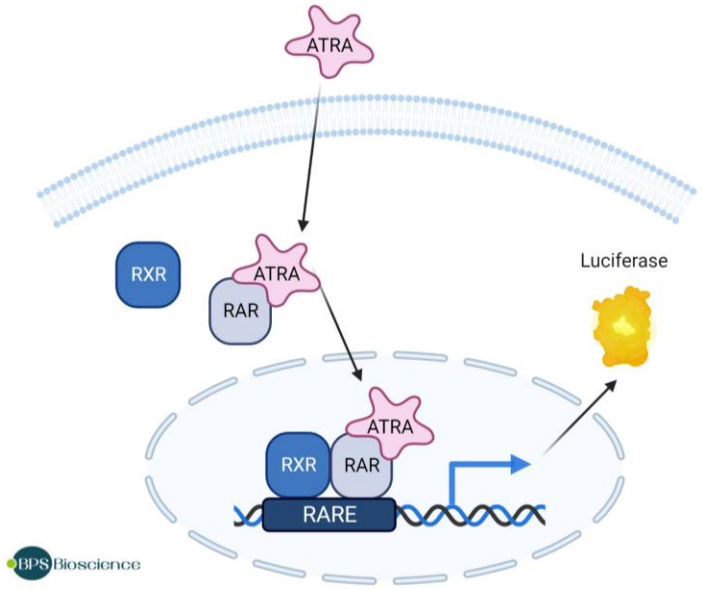

Figure 1: Activation of retinoid acid-dependent luciferase reporter in the RARα/RARα Luciferase Reporter HEK293 Cell Line.

Background

RAR (Retinoic Acid Receptor) belongs to a family of nuclear receptors and has three subtypes: RARa, RARb, and RARg. RAR heterodimerizes with RXR (Retinoid X Receptor) 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 (ATRA) or *9-cis* retinoic acid, the RAR/ RXR heterodimer binds to the retinoic acid response elements in the promoter region of target genes. This recruits coactivator proteins, leading to transcription and expression of the downstream target genes. Retinoic acid (a derivative of Vitamin A) signaling plays critical roles in embryonic and immune system development. Mutations in RARα can lead to Acute Promyelocytic Leukemia (APL), characterized by the accumulation of progenitor cells from the blood lineage (promyelocytes), and other cancer types. Use of RA may prove beneficial in cancer therapy.

Application

- Monitor RARa-regulated pathway activity.
- Screen RARa agonists and antagonists.

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 supplemented with 10% FBS and 1% Penicillin/Streptomycin.

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

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
Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once 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 in Thaw Medium 6 to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 48-72 hours in culture, check for cell viability, change 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 reach 100% confluency. Switch to Growth Medium 6A for passage.

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 0.25% Trypsin/EDTA following volumes recommended for the cell vessel being used.

2. Once the cells have detached, add Growth Medium 6A and transfer to a tube.

3. Spin down 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. After detachment, spin down the cells at 300 *x g* for 5 minutes.

2. Remove the medium and resuspend the cell pellet in 4°C Cell Freezing Medium (BPS Bioscience #79796) at a density of ~2 x 10^6 cells/ml.

3. 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.

4. 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.

Validation Data
Dose response of RARα Reporter – HEK293 Recombinant Cell Line to *all-trans* retinoic acid (ATRA)

Assay Protocol

- The following assay is designed for 96-well format. To perform the assay in different tissue culture formats, cell number and reagent volume should be scaled appropriately.
- All samples and controls should be performed in triplicate.
- This assay should include "Cell-Free Control", "Unstimulated Control" and "Test Compound" conditions.

Assay Medium:
Assay Medium 6A.

1. 24 hours before plating the cells remove Growth Medium 6A and replace with Assay Medium.

2. Dissociate the cells and seed ~30,000 cells/well in 90 µl of Assay Medium in a 96-well white clear-bottom plate.

3. Add 90 µl of Assay Medium to "Cell Free Control" wells (for determining background luminescence).

4. Prepare ATRA (10 µl/ well): start by preparing a stock solution of ATRA in 100% DMSO at 2000-fold the highest desired concentration. The final volume is 100 µl.

   Dilute 200-fold in Assay Medium to prepare an intermediate 10x stock solution with 0.5% DMSO.

   Prepare a serial dilution of ATRA in 0.5% DMSO in Assay Medium at concentrations 10-fold higher than the desired final concentrations. Prepare Assay Medium with 0.5% DMSO (Diluent Solution) to use in controls (Diluent Solution).

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

5. Add 10 µl of diluted ATRA to each well.

6. Add 10 µl of Diluent Solution to the "Unstimulated Control" wells "Cell-Free Control" wells (background).

Component	Cell Free Control	Unstimulated Control	Test Compound
Assay Medium	90 µl	-	-
Cell suspension	-	90 µl	90 µl
Diluted ATRA	-	-	10 µl
Diluent Solution	10 µl	10 µl	-
Total	**100 µl**	**100 µl**	**100 µl**

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

8. Add 100 µl of ONE-Step™ Luciferase reagent per well and gently agitate at Room Temperature for ~15 minutes.

9. Measure the luminescence using a luminometer.

10. Subtract average background luminescence (cell-free control wells) from the luminescence reading of all wells. Fold induction of RARα luciferase reporter expression can be calculated by dividing the ATRA-stimulated Luminescence with the Unstimulated Control Luminescence.

$$Fold\ induction = \frac{ATRA\ stimulated\ Lumin. - Background}{Unstimulated\ Lumin. - Background}$$

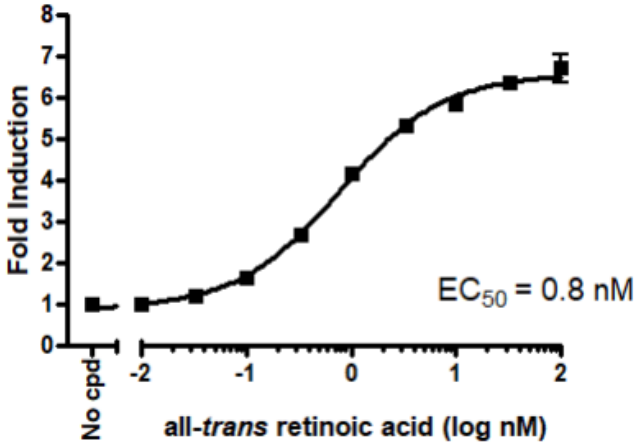

Figure 2: Dose response of RARα Luciferase Reporter HEK293 Cell Line to all-trans retinoic acid (ATRA).
Cells were incubated with increasing amounts of ATRA. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. Results are expressed as Fold Induction (calculated against the "Unstimulated Cells Control", which was set to 1).

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):440-50.
Allenby J., *et al*., 1993 *Proc Natl Acad Sci USA* 90 (1):30-4.
Peng Z., *et al*., 2023 *J Immunotherapy Cancer* 11 (3): e006325.

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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*
RARγ Reporter - HEK293 Recombinant Cell Line	60604	2 vials
RARβ Reporter – HEK293 Recombinant Cell Line	60603	2 vials
RARα Reporter Cellular Assay Pack	79322	2 vials
RARβ Reporter Cellular Assay Pack	79323	2 vials
RARγ Reporter Cellular Assay Pack	79324	2 vials

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