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Data Sheet

Transfection Collection™ - RARγ Reporter Cellular Assay Pack
Catalog #: 79324

Background
Retinoic acid receptor (RAR) belongs to a family of nuclear receptors and has three subtypes, RARα, RARβ, and RARγ. 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 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.

Description
The *RARγ* Reporter Cellular Assay Pack provides all the key reagents required to monitor the activity of retinoic acid receptor gamma (RARγ). The pack contains the *RARγ Reporter (Luc)-HEK293 Cell Line*, a luciferase reporter cell line that contains a firefly luciferase gene under the control of retinoic acid response elements stably integrated into HEK293 cells along with full length human RARα (GenBank Accession No. P13631-1). This cell line is functionally validated for the response to the stimulation of all-*trans* retinoic acid, and the expression of RARγ is confirmed by Western blotting.

Additionally, the pack includes cell culture medium (Thaw Medium 6) that has been optimized for use with HEK293 cells. Thaw Medium 6 includes 10% fetal bovine serum and 1% Pen/Strep. Finally, the pack provides the ONE-Step™ Luciferase Detection System. This reagent provides highly sensitive, stable detection of firefly (*Photinus pyralis*) luciferase activity. The ONE-Step luciferase reagent can be used directly in cells in growth medium, and can be detected with any luminometer; automated injectors are not required.

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

Storage
Immediately upon receipt, store in liquid nitrogen.

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Components

Cat. #	Component	Amount	Storage
60604	RARγ Reporter (Luc) - HEK293 Cell Line	2 vials*	liquid nitrogen
60690-1	ONE-Step Luciferase Buffer (**Component A**)	10 ml	-20°C
	ONE-Step Luciferase Reagent Substrate, 100x **(Component B)**	100 µl	-20°C *Protect from light*
60183	Thaw Medium 6	100 ml	+4°C

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

General Culture Conditions

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

Complete Growth Medium: Thaw Medium 6 and 400 µg/ml of Geneticin (G418; Invitrogen, #11811031), 1 µ g/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 (Thaw Medium 6 plus Puromycin and Hygromycin). If culturing cells in medium from other vendors, it may be required 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, and transfer to a tube containing 10 ml of Thaw Medium 6 **(no Geneticin, Puromycin, and Hygromycin)**. Spin down cells, resuspend cells in pre-warmed Thaw Medium 6 **(no Geneticin, Puromycin, and Hygromycin)**, and transfer resuspended cells to a T25 flask and culture in 37°C CO_2 incubator. At first passage, switch to complete growth medium **(contains 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, add complete growth medium and 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.

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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.

Mycoplasma testing
The cell line has been screened using the PCR-based VenorGeM® *Mycoplasma* Detection kit (Sigma-Aldrich, #MP0025) to confirm the absence of *Mycoplasma* species.

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-Aldrich, #R2625): stock solution in DMSO
- 96-well tissue culture treated white clear-bottom assay plate (Corning, # 3610)
- Luminometer

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 complete growth medium from RARγ Reporter (Luc)-HEK293 cells and replace with Thaw Medium 6 for 24 hours.

2. Harvest RARγ Reporter (Luc)-HEK293 cells and seed cells in 40 µl of Thaw Medium 6 at a density of ~30,000 cells per well in a 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 ATRA-stimulated 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°C in a CO_2 incubator for ~ 16 to 24 hours.

5. Perform the luciferase detection assay using the ONE-Step™ Luciferase Assay System according to the protocol below:

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Luciferase Detection Procedure

6. Thaw Luciferase Reagent Buffer (**Component A**) by placing the reagent in a room temperature water bath. Equilibrate the buffer to room temperature and mix well before use. Note: Luciferase Reagent Buffer must be at ~ room temperature (20- 25°C) before use.
7. Calculate the amount of Luciferase Reagent needed for the experiment (**Component A** + **Component B**). Immediately prior to performing the experiment, prepare the luciferase assay working solution by diluting Luciferase Reagent Substrate (**Component B)** into Luciferase Reagent Buffer (**Component A)** at a 1:100 ratio and mix well. Avoid exposing to excessive light. *Only use enough of each component for the experiment, remaining **Component A** and **Component B** should be stored separately at* -20°C.
8. Remove multi-well plate containing mammalian cells from incubator. *Note: plates must be compatible with luminescence measurement with luminometer being used.*
9. Add 50 µl of luciferase assay working solution (**Component A** + **Component B**) to the culture medium in each well.
10. Gently rock the plates for ≥15 minutes at room temperature. Measure firefly luminescence using a luminometer.

The signal under these conditions will be stable for more than 2 hours at room temperature. For maximal light intensity, measure samples within 1 hour of reagent addition.

Data Analysis: Background luminescence is a characteristic of luminometer performance, therefore, background luminescence must be subtracted from all readings for accuracy. Obtain the background-subtracted luminescence by subtracting the 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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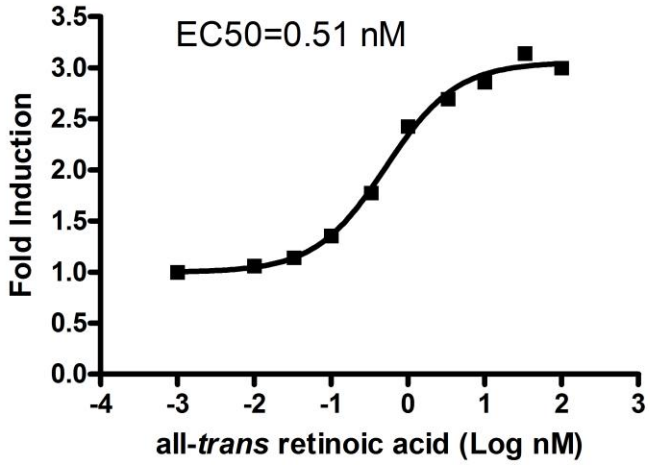
Figure 1. Dose response of RARγ Reporter (Luc) - HEK293 cells to all-*trans* retinoic acid (ATRA). Results were shown as fold induction of RAR luciferase reporter expression.



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.

License Disclosure

Purchase of this cell line grants you with a 10-year license to use this cell line in your immediate laboratory, for research use only. This license does not permit you to share, distribute, sell, sublicense, or otherwise make the cell line available for use to other laboratories, departments, research institutions, hospitals, universities, or biotech companies. The license does not permit use of this cell line in humans or for therapeutic or drug use. The license does not permit modification of the cell line in any way. Inappropriate use or distribution of this cell line will result in revocation of the license and result in an immediate cease of sales and distribution of BPS products to your laboratory. BPS does not warrant the suitability of the cell line for any particular use, and does not accept any liability in connection with the handling or use of the cell line. Modifications of this cell line, transfer to another facility, or commercial use of the cells may require a separate license and additional fees**;** contact *sales@bpsbioscience.com* for details. Publications using this cell line should reference BPS Bioscience, Inc., San Diego.

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Refills

Product	Cat. #	Size
RARγ Reporter - HEK293 Reporter Cell Line	60604	2 vials
ONE-Step Luciferase Assay Detection System	60690-1	10 ml
ONE-Step Luciferase Assay Detection System	60690-2	100 ml
ONE-Step Luciferase Assay Detection System	60690-3	1 L
Thaw Medium 6	60183	100 ml

Related Products

Product	Cat. #	Size
RARα Reporter (Luc) - HEK293 Cell Line	60503	2 vials
RARβ Reporter - HEK293 Recombinant Cell Line	60603	2 vials
Anti–RARA polyclonal antibody	25310	100 µl
Thaw Medium 1	60187	100 ml
NcoR2 (SMRT), GST-tag	50020	50 µg