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Data Sheet

Transfection Collection™ - RAR γ Reporter Cellular Assay Pack **Catalog #: 79324**

Background

Retinoic acid receptor (RAR) belongs to a family of nuclear receptors and has three subtypes, RAR α , RAR β , and RAR γ . 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 RAR γ Reporter Cellular Assay Pack provides all the key reagents required to monitor the activity of retinoic acid receptor gamma (RAR γ). The pack contains the RAR γ 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 RAR α (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 RAR γ 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 RAR γ -regulated pathway activity
- Screen agonists or antagonists of RAR α .

Storage

Immediately upon receipt, store in liquid nitrogen.

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Components

Cat. #	Component	Amount	Storage
60604	RAR γ 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 μ l	-20°C <i>Protect from light</i>
60183	Thaw Medium 6	100 ml	+4°C

*Each vial contains $\sim 2 \times 10^6$ 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 μ g/ml of Geneticin (G418; Invitrogen, #11811031), 1 μ g/ml of Puromycin (Hyclone, #SV30075.01), and 100 μ g/ml Hygromycin (Hyclone, #SV30070.01).

Cells should be maintained at 37°C with 5% CO₂ 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 CO₂ in the incubator depending on the NaHCO₃ 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 CO₂ 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 RAR γ Reporter (Luc)-HEK293 cells and replace with Thaw Medium 6 for 24 hours.
2. Harvest RAR γ Reporter (Luc)-HEK293 cells and seed cells in 40 μ l 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 μ l of ATRA solution to each ATRA-stimulated well. The final DMSO concentration is 0.1%.
Add 10 μ l of assay medium with 0.5% DMSO to the unstimulated control wells.
Add 50 μ l 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 CO₂ 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

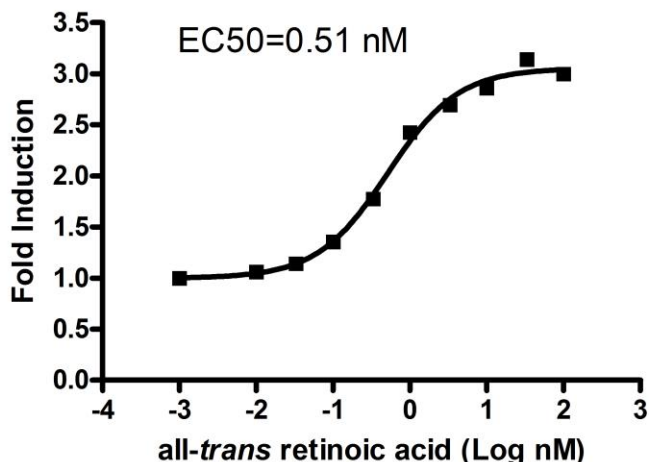
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Figure 1. Dose response of RAR γ Reporter (Luc) - HEK293 cells to all-*trans* retinoic acid (ATRA). Results were shown as fold induction of RAR luciferase reporter expression.



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.

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Refills

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
RAR γ 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

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
RAR α Reporter (Luc) - HEK293 Cell Line	60503	2 vials
RAR β Reporter - HEK293 Recombinant Cell Line	60603	2 vials
Anti-RARA polyclonal antibody	25310	100 μ l
Thaw Medium 1	60187	100 ml
NcoR2 (SMRT), GST-tag	50020	50 μ g

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