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## Data Sheet

### ***RAR $\beta$ Reporter Cellular Assay Pack*** **Catalog #: 79323**

#### **Background**

Retinoic acid receptor (RAR) belongs to a family of nuclear receptors and has three subtypes, RAR $\alpha$ , RAR $\beta$ , and RAR $\gamma$ . 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 $\beta$  Reporter Cellular Assay Pack* provides all the key reagents required to monitor the activity of retinoic acid receptor beta (RAR $\beta$ ). The pack contains the *RAR $\beta$  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 $\alpha$  (GenBank Accession No. P10826-2). This cell line is functionally validated for the response to the stimulation of all-*trans* retinoic acid, and the expression of RAR $\beta$  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 $\beta$ -regulated pathway activity
- Screen agonists or antagonists of RAR $\alpha$ .

#### **Storage**

Immediately upon receipt, store in liquid nitrogen.

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## Components

Cat. #	Component	Amount	Storage
60603	RAR $\beta$ Reporter (Luc) - HEK293 Cell Line	2 vials*	liquid nitrogen
60690-1	ONE-Step Luciferase Buffer ( <b>Component A</b> )	10 ml	-20°C
	ONE-Step Luciferase Reagent Substrate, 100x ( <b>Component B</b> )	100 $\mu$ 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 (BPS Bioscience, #60813):** 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  $\mu$ g/ml of Geneticin (G418; Invitrogen, #11811031), 1  $\mu$ g/ml of Puromycin (Hyclone, #SV30075.01), and 100  $\mu$ g/ml Hygromycin (Hyclone, #SV30070.01).

Cells should be maintained at 37°C with 7% CO<sub>2</sub> 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<sub>2</sub> in the incubator depending on the NaHCO<sub>3</sub> 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<sub>2</sub> 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 beta Reporter (Luc) - HEK293 cells to all-*trans* retinoic acid (ATRA)**

1. One day before plating the cells, remove the complete growth medium from RAR $\beta$  Reporter (Luc)-HEK293 cells and replace with Thaw Medium 6 for 24 hours.
2. Harvest RAR $\beta$  Reporter (Luc)-HEK293 cells and seed cells in 40  $\mu$ 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  $\mu$ l of ATRA solution to each ATRA-stimulated well. The final DMSO concentration is 0.1%.  
Add 10  $\mu$ l of assay medium with 0.5% DMSO to the unstimulated control wells.  
Add 50  $\mu$ 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 CO<sub>2</sub> 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

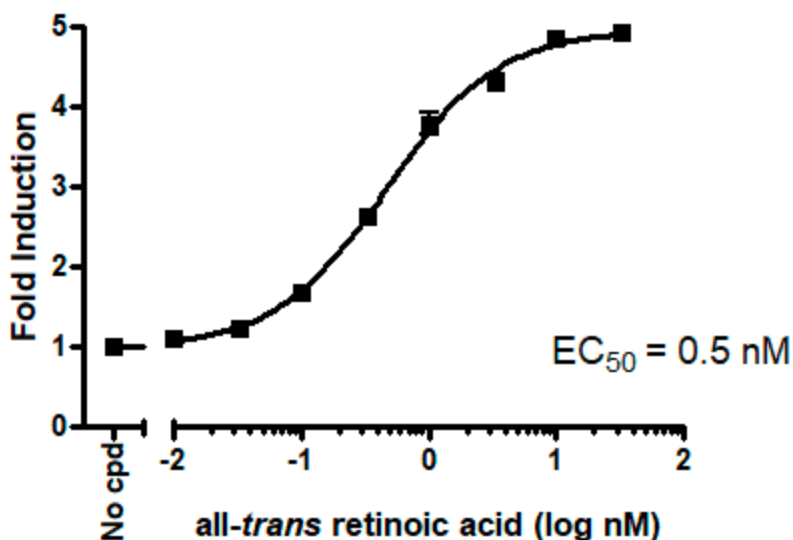
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**Figure 1. Dose response of RAR $\beta$  Reporter (Luc) - HEK293 cells to all-*trans* retinoic acid (ATRA). Results were shown as fold induction of RAR luciferase reporter expression.**



## 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 $\beta$ Reporter - HEK293 Recombinant Cell Line	60603	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 $\alpha$ Reporter (Luc) - HEK293 Cell Line	60503	2 vials
RAR $\gamma$ Reporter - HEK293 Reporter Cell Line	60604	2 vials
Anti-RARA polyclonal antibody	25310	100 $\mu$ l
Thaw Medium 1	60187	100 ml
NcoR2 (SMRT), GST-tag	50020	50 $\mu$ g

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