Description

Estrogen Luciferase Reporter T47D Cell Line is a human breast cancer, ERα (estrogen receptor alpha)-positive luminal A cell line, with a stably integrated Firefly luciferase reporter under the control of an estrogen response element (ERE). This cell line monitors the activity of the ER signaling pathway.

This cell line has been validated in cellular assays involving the inhibition of 17β-Estradiol-induced reporter activation by ER antagonists such as Vepdegestrant (ARV-471), Elacestrant (RAD1901), and Fulvestrant.

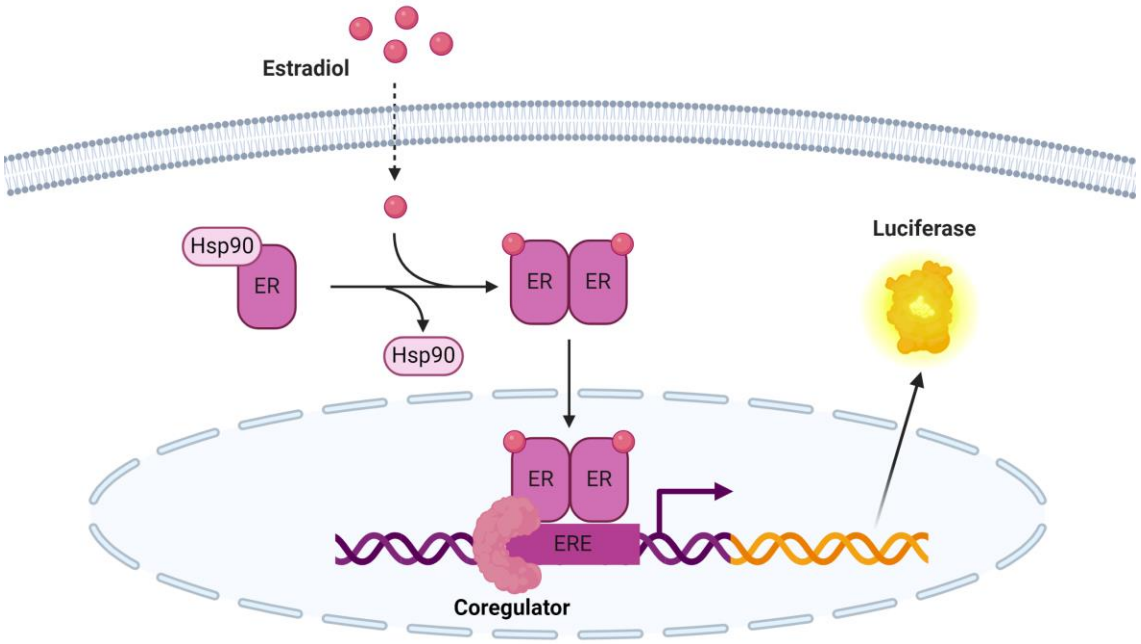

Figure 1: Mechanism of action of Estrogen Luciferase Reporter T47D Cell Line in response to 17β-Estradiol.

Background

ER (estrogen receptor) exists as two nuclear ER isoforms (ERα and ERβ) and a membrane associated ER (GPER1) one and responds to estrogen. Estrogen is a hormone produced mainly in the ovaries, but also in the adipose tissue, and plays a role in reproduction, bone density, inflammation, and others. Estrogen can penetrate the cell membrane and bind to ERα, which dimerizes and translocates to the nucleus, where it associates with transcriptional co-activators and binds to ERE (estrogen responsive elements). In addition to this genomic role, ER also plays non genomic roles by directly activating cellular signaling pathways. About 60% of breast cancer (BC) cases are ERα-positive and are classified as luminal BC. Patients with luminal BC have several therapeutic strategies available to them: hormone therapy, SERM (selective estrogen receptor modulators), AI (aromatase inhibitors), SERD (selective estrogen receptor degraders) and LHRH (luteinizing hormone-releasing hormone). However, about half of the metastatic therapy-resistant BC result from ERα mutations, with at least 62 being known. New therapeutic avenues are thus needed for the successful treatment of this BC patient population.

Application

- Screen and characterize modulators of the estrogen receptor signaling pathway.
- Evaluate the efficacy of potential drug candidates for ER-directed therapies.

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

T47D, human breast carcinoma epithelial cell line, 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 2	BPS Bioscience #60184
Growth Medium 2M	BPS Bioscience #78181
Insulin Solution	MilliporeSigma #I9278

Materials Used in Cellular Assay

Name	Ordering Information
Assay Medium 2C	BPS Bioscience #78544
Assay Medium 2F	BPS Bioscience #82784
17β-Estradiol	MilliporeSigma #E8875
Vepdegestrant (ARV-471)	MedChem #HY-138642
Elacestrant (RAD1901)	MedChem #HY-19822A60
Fulvestrant	MedChem #HY-13636
Clear-bottom, white 96-well tissue culture-treated plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Trypsin-EDTA (0.5%), no phenol red	FisherScientific #15-400-054
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 and Functional Cellular Assay

Complete Thaw Medium 2:

Thaw Medium 2 (BPS Bioscience #60184) + 10 µg/ml Insulin (MilliporeSigma#I9278): RPMI 1640 (ATTC modification) medium supplemented with 10% FBS, 1% Penicillin/Streptomycin, and 10 µg/ml Insulin.

Complete Growth Medium 2M:

Growth Medium (BPS Bioscience #78181) + 10 µg/ml Insulin (MilliporeSigma#I9278): RPMI 1640 (ATCC modification) medium supplemented with 10% FBS, 1% Penicillin/Streptomycin, 1 µg/ml of Puromycin and 10 µg/ml Insulin.

Note: the final concentration of 10 µg/ml Insulin (MilliporeSigma#I9278) must be added to Thaw Medium 2 and Growth Medium 2M for cell culture.

Assay Medium 2C (BPS Bioscience #78544):

RPMI 1640 (ATCC modification) medium (no phenol red) supplemented with 10% charcoal-stripped FBS and 1% Penicillin and Streptomycin.

Assay Medium 2F (BPS Bioscience #82784):

RPMI 1640 (ATCC modification) medium (no phenol red) supplemented with 0.5% charcoal-stripped FBS.

Cell Culture Protocol

Note: T47D 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. 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 Complete Thaw Medium 2.

   Note: 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 Complete Thaw Medium 2.

3. Transfer the resuspended cells to a T25 flask or T75 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 Complete Thaw Medium 2 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 Complete Growth Medium 2M.

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.

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

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Complete Growth Medium 2M.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1/3 to 1/6 once or twice per week.

   Note: Just after thawing and when cells are at low density, the cells may grow at a slower rate. It is recommended to split the cells at a 1/3 ratio in those cases. After several passages, the cell growth rate increases, and the cells can be split using a higher ratio.

Cell Freezing

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.

2. Once the cells have detached, add Complete Growth Medium 2M 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.

Validation Data

The following assays were designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.

A. Dose response to 17β-Estradiol by Estrogen Luciferase Reporter T47D Cell Line

- This experiment measures the effect of a compound on reporter activation.
- All conditions should be performed in triplicate.
- The assay should include “Stimulated”, “Cell-Free Control” and “Unstimulated Control” conditions.

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.5% Trypsin/EDTA, no phenol red.

Note: It is recommended to use phenol red free trypsin at this step.

2. Once the cells have detached, add Assay Medium 2C and transfer to a tube.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium/trypsin and resuspend the cells in Assay Medium 2C at 40,000 cells/ml.

4. Seed Estrogen Luciferase Reporter T47D cells at a density of ~4,000 cells/well in 100 µl of Assay Medium 2C into a clear-bottom, white 96-well plate. Leave a few wells empty as "Cell-Free Control" wells (as background luminescence control).

Note: Cells were seeded in Assay Medium 2C but stimulated in Assay Medium 2F.

5. Incubate the plate at 37°C in a 5% CO_2 incubator for 24 hours.

6. Prepare the compound of interest at the concentration to be tested, in Assay Medium 2F (100 µl/well).

7. Carefully remove the medium from all wells.

8. Add 100 µl of the compound to be tested to the "Stimulated" wells.

9. Add 100 µl of Assay Medium 2F to the "Unstimulated Control" (to determine the unstimulated luminescence from Estrogen Luciferase Reporter T47D cells) and "Cell-Free Control" wells (to determine background luminescence).

10. Incubate the plate at 37°C with 5% CO_2 for ~24 hours.

11. Add 100 µl of ONE-Step™ Luciferase reagent to each well and rock at Room Temperature (RT) for ~15 to 30 minutes.

12. Measure luminescence using a luminometer.

13. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The fold induction of luciferase reporter expression is the average background-subtracted luminescence of stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ \ Stimulated\ Wells - avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells - avg.background}$$

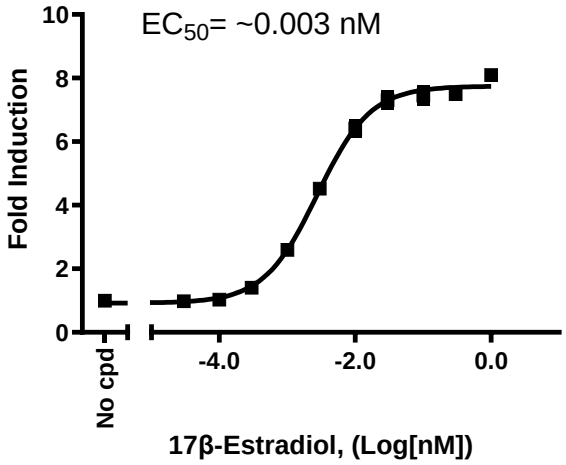

Figure 2: Reporter activation in Estrogen Luciferase Reporter T47D Cell Line in response to 17β-Estradiol.
Estrogen Luciferase Reporter T47D cells were seeded at 4,000 cells/well in a 96-well plate in Assay Medium 2C for 24 hours, and then stimulated with increasing concentrations of 17β-Estradiol in Assay Medium 2F for 24 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter activity in relation to the activity of cells without agonist.

B. Inhibition of 17β-Estradiol-induced reporter activation by ER antagonists in Estrogen Luciferase Reporter T47D Cell Line.

- The effect of an antagonist compound is measured against agonist activation.
- The assays should include "Stimulated, No Antagonist", "Unstimulated, No Antagonist", "Cell-Free Control", and "Stimulated, Antagonist" conditions.

1. Seed Estrogen Luciferase Reporter T47D cells at a density of 4,000 cells/well in 100 µl of Assay Medium 2C, into a white clear-bottom 96-well cell culture plate, as described in A.

2. Incubate the cells at 37°C with 5% CO_2 for 24 hours.

3. Prepare a three-fold serial dilution of ER antagonists in Assay Medium 2F (50 µl/well) at concentrations 2-fold higher than the desired final concentrations.

4. Remove the cell culture medium from the cells.

5. Add 50 µl of each antagonist dilution to the "Stimulated, Antagonist" wells.

6. Add 50 µl of Assay Medium 2F to the "Stimulated, No Antagonist" and "Unstimulated, No Antagonist" wells.

7. Incubate the cells at 37°C in 5% CO_2 for one hour.

8. Prepare 17β-Estradiol in Assay Medium 2F at a concentration of 0.06 nM (the final concentration will be 0.03 nM) (50 µl/well).

9. Add 50 µl of diluted 17β-Estradiol to the "Stimulated, Antagonist" and "Stimulated, No Antagonist" wells.

10. Add 50 µl of Assay Medium 2F to the "Unstimulated, No Antagonist" (for determining ER basal activity) wells.

11. Add 100 µl of Assay Medium 2F to "Cell-Free Control" wells (for determining background luminescence).

12. Incubate at 37°C in 5% CO_2 for 24 hours

13. Add 100 µl/well of ONE-Step™ Luciferase Assay reagent.

14. Incubate with gentle agitation at RT for ~15 to 30 minutes.

15. Measure luminescence using a luminometer.

16. Data Analysis: Subtract the background luminescence from the luminescence reading of all conditions. The percent luminescence is the background-subtracted luminescence of antagonist-treated cells divided by the background-subtracted luminescence of agonist-activated control cells ("Stimulated, No Antagonist" condition), multiplied by 100. The result of 17β-Estradiol-stimulated cells in the absence of antagonist is set at 100%.

$$Percent\ Luminescence = \left(\frac{luminescence\ of\ Antagonist\ treated\ cells - avg.background}{luminescence\ of\ Stimulated,\ \ No\ Antagonist\ cells - avg.background}\right) \text{x}100$$

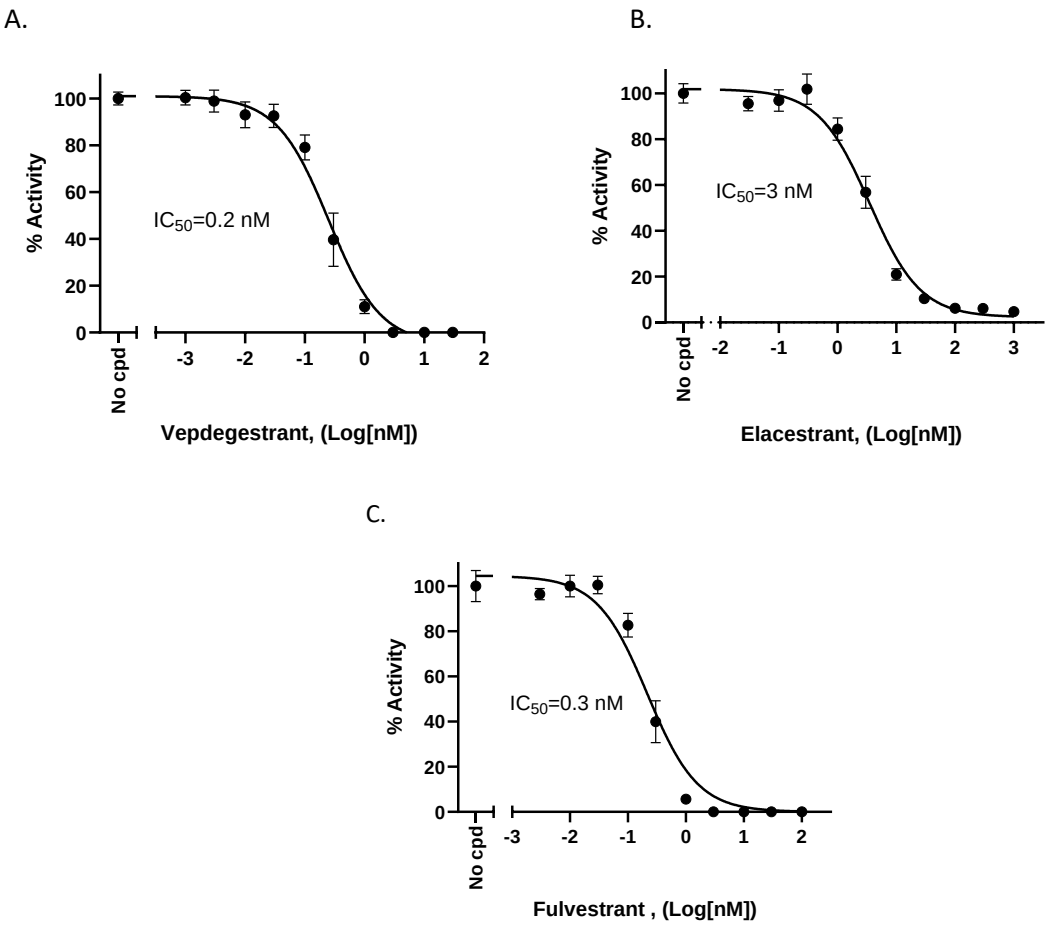

Figure 3. Inhibition of 17β-Estradiol induced reporter activity by Vepdegestrant, Elacestrant, Fulvestrant in Estrogen Luciferase Reporter T47D Cell Line.
Cells were incubated with increasing concentrations of the Estrogen receptor antagonists Vepdegestrant, Elacestrant, Fulvestrant for 1 hour, followed by stimulation with 0.03 nM 17β-Estradiol for 24 hours. Luciferase activity was measured using ONE-Step™ Luciferase Assay System. Results are shown as percentage of reporter activity (compared to cells stimulated by 17β-Estradiol without antagonist).

Data shown is representative.

References

Juliette L., *et al.*, 1999 *Toxicological Sciences* 48: 55-66.
Yu S., *et al.*, 2017 *Biochemical and Biophysical Research Communications* 486(3): 752-758.
Clusan L., *et al.*, 2023 *Int J Mol Sci* 24(7): 6834.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
Androgen Luciferase Reporter 22RV1 Cell Line	78972	2 vials
ER Luciferase Reporter Lentivirus	78764	500 µl x 2
AR Luciferase Reporter Lentivirus	78763	500 µl x 2

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