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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

Androgen Luciferase Reporter 22RV1 Cell Line is a human 22Rv1 cell line with a stably integrated Firefly luciferase reporter under the control of an androgen response element. This cell line monitors the activity of the androgen receptor signaling pathway.

This cell line has been validated in cellular assays involving the inhibition of 5α-Dihydrotestosterone (5-DHT)-induced reporter activation by AR (androgen receptor) antagonists such as Enzalutamide, Bicalutamide, Mifepristone and ARCC-4.

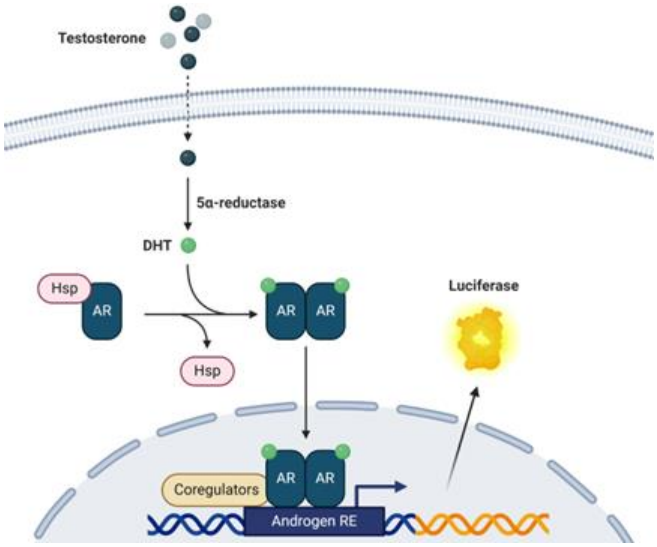

Figure 1: Mechanism of action of Androgen Luciferase Reporter 22RV1 Cell Line in response to testosterone.

Background

AR (androgen receptor), also known as NR3C4 (nuclear receptor family 3, group C, member 4) is a nuclear receptor involved in regulating gene expression. It is activated by testosterone and dihydrotestosterone, and it has been linked to prostate cancer. Prostate cancer is the most frequently diagnosed cancer and the second-leading cause of cancer death in American men. AR remains functional and is expressed in nearly all primary prostate cancers, with endocrine therapy aiming at reducing serum androgens and inhibiting AR. The androgen-induced transcriptional activation of AR is modulated by the interaction of AR with coregulators and by phosphorylation of AR and AR coregulators in response to growth factors. However, prostate cancer can almost always adapt to survive under castration levels of androgen or to AR inhibition. Castration resistance may involve AR point mutations, overexpression, changes in androgen biosynthesis, expression of constitutively active AR splice variants (active in the absence of ligand binding), and changes in androgen cofactors. As AR activity remains important in the progression of all stages of prostate cancer, AR continues to be an attractive molecular target of drugs against prostate cancer. 22Rv1 cell line is derived from a human prostate cancer xenograph propagated in mice and it is known to form tumors in nude mice. It is mostly dihydrotestosterone-independent for growth but responds to EGF (epidermal growth factor). They express androgen receptor (AR) that responds to androgen stimulation, making it one of the few available cellular models for prostate cancer studies.

Application

- Screen and characterize modulators of the androgen receptor signaling pathway.
- Evaluate potential drug candidates in AR-directed therapies and their therapeutic efficacy.

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

22Rv1, human prostate 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 Medium2M	BPS Bioscience #78181

Materials Used in Cellular Assay

Name	Ordering Information
Assay Medium 2C	BPS Bioscience #78544
5α-Dihydrotestosterone (5-DHT)	Sigma-Aldrich #D-073
Enzalutamide	MedChem #HY-70002
Bicalutamide	MedChem #HY-14249
Mifepristone	Cayman #10006317
ARCC-4	MedChem #HY-130492
Clear-bottom, white 96-well tissue culture-treated 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 and Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience #60184):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2M (BPS Bioscience #78181):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 µg/ml of Puromycin.

Assay Medium 2C (BPS Bioscience #78544):
RPMI-1640 medium (no phenol red) supplemented with 10% charcoal-stripped FBS and 1% Penicillin and Streptomycin.

Cell Culture Protocol

Note: 22Rv1 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 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 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 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 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 0.05% Trypsin/EDTA.

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

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1/3 to 1/6 weekly 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 1/4 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 0.05% Trypsin/EDTA.

2. Once the cells have detached, add 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 of 5-DHT in Androgen Luciferase Reporter 22RV1 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. Seed Androgen Luciferase Reporter 22RV1 cells at a density of ~20,000 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).

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

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

4. Carefully remove the medium from all wells.

5. Add 100 µl of the compound to be tested to the “Stimulated” wells.

6. Add 100 µl of Assay Medium 2C to the “Unstimulated Control” (to determine the unstimulated luminescence from Androgen Luciferase Reporter 22RV1 cells) and “Cell-Free Control” wells.

7. Incubate the plate at 37°C with 5% CO_2 for ~16 hours.

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

9. Measure luminescence using a luminometer.

10. 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.

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

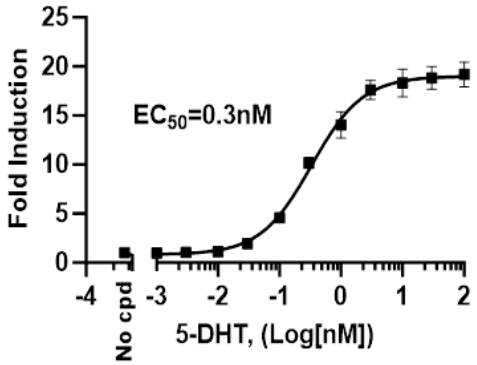

Figure 2: Reporter activation in Androgen Luciferase Reporter 22RV1 Cell Line in response to 5-DHT.
Androgen Luciferase Reporter 22RV1 cells were incubated with increasing concentrations of 5-DHT for 16 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter activity in relation to the activity of cells without agonist.

B. Inhibition of 5-DHT-induced reporter activation by AR antagonists in Androgen Luciferase Reporter 22RV1 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 Androgen Luciferase Reporter 22RV1 cells at a density of 20,000 cells/well in 100 µl of Assay Medium 2C, into a white clear-bottom 96-well cell culture plate. Keep wells without cells as "Cell-Free Control" (for determining the background luminescence).

2. Incubate the cells at 37°C with 5% CO_2 overnight.

3. Prepare a three-fold serial dilution of AR antagonists in Assay Medium 2C (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 2C to the “Stimulated, No Antagonist” and “Unstimulated, No Antagonist” wells.

7. Incubate the cells at 37°C in 5% CO_2 for the desired length of time.

8. Prepare 5-DHT in Assay Medium 2C at a concentration of 4 nM (the final concentration will be 2 nM) (50 µl/well).

9. Add 50 µl of diluted 5-DHT to the “Stimulated, Antagonist” and “Stimulated, No Antagonist” wells.

10. Add 50 µl of Assay Medium 2C to the “Unstimulated, No Antagonist” (for determining AR basal activity) wells.

11. Add 100 µl of Assay Medium 2C to “Cell-Free Control” wells.

12. Incubate at 37°C in 5% CO_2 overnight.

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 5-DHT-stimulated cells in the absence of antagonist is set at 100%.

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

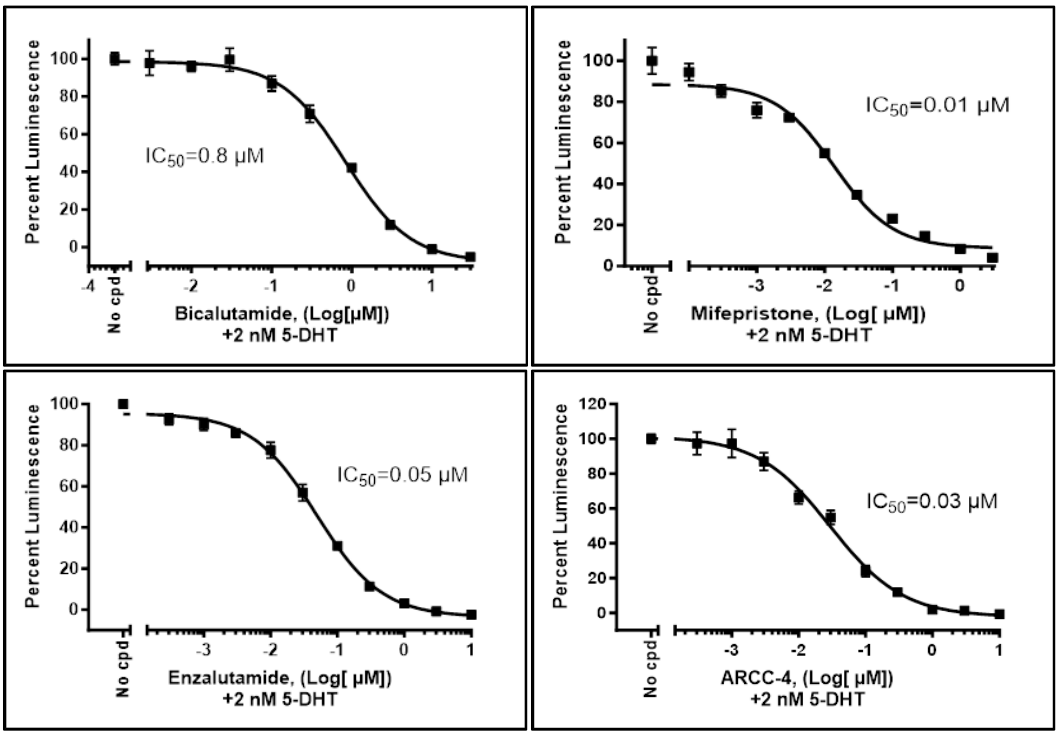

Figure 3. Inhibition of 5-DHT-induced reporter activity by Enzalutamide, Bicalutamide, Mifepristone and ARCC-4 in Androgen Luciferase Reporter 22RV1 Cell Line.
Cells were incubated with increasing concentrations of the androgen receptor antagonists Enzalutamide, Bicalutamide, Mifepristone for 1 hour and ARCC-4 for 6 hours. This was followed by stimulation with 2 nM 5-DHT overnight. Luciferase activity was measured using ONE-Step™ Luciferase Assay System. Results are shown as percentage of reporter activity (compared to cells stimulated by 5-DHT without antagonist).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Heinlein C.A. and Chang C., 2004 *Endocr Rev.* 25(2):276-308.
Lonergan P.E., and Tindall D.J., 2011 *J Carcinog.* 10:20.
Salami J., *et al.*, 2018 *Commun Biol* 1:100.

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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*
HIPK3 Kinase Assay Kit	78382	96 reactions
Anti-PSMA-Anti-CD3 IgG format Bispecific Antibody	101242	50 µg/100 µg
PSMA (FOLH1) – CHO Cell Line (High, Medium or Low Expression)	79641	2 vials
PSMA Lentivirus	78726	500 µl x 2
Anti-PSMA Antibody	101695	50 µg
Anti-PSMA Antibody, PE-Labeled	101976	25 µg/100 µg

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