

# Product Sheet

## H\_AR Reporter 293 Cell Line

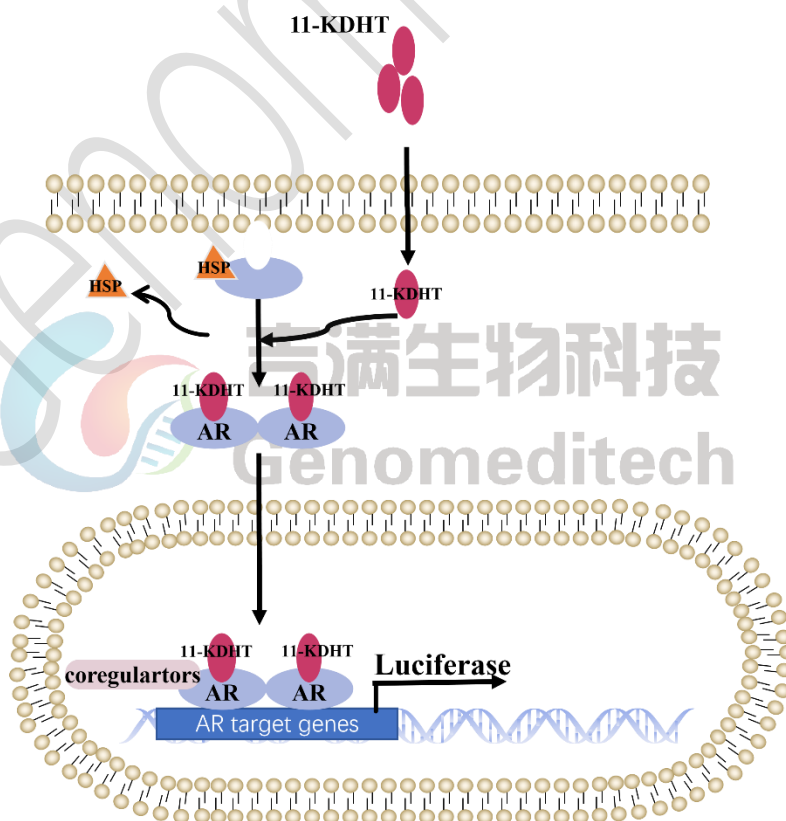
Catalog number: GM-C45341

Version 3.3.1.260617

Androgen receptor (AR), also known as NR3C4 and encoded by the AR gene, is a ligand-activated transcription factor belonging to the steroid receptor subfamily of the nuclear receptor superfamily, predominantly expressed in androgen-responsive tissues such as the prostate, testis, and skeletal muscle. As the specific receptor for testosterone and dihydrotestosterone (DHT), AR plays a pivotal role in male sexual differentiation, prostate development, and bone metabolism, making it a critical therapeutic target for prostate cancer, androgenetic alopecia, and muscle-wasting disorders.

Upon binding of DHT or testosterone, AR undergoes conformational change, dissociates from heat shock proteins, dimerizes, and translocates into the nucleus, where it binds to androgen response elements (AREs) to activate target gene transcription. driver of prostate cancer progression.

H\_AR Reporter Cell Line is a clonal stable cell line constructed using lentiviral technology, constitutive expression of the AR gene, along with signal-dependent expression of a luciferase reporter gene under the control of androgen response elements (AREs). When DHT or other AR agonists bind to AR, it undergoes nuclear translocation and binds to AREs, activating the expression of luciferase. The luciferase activity measurement indicates the activation level of the AR signaling pathway and can thus be used to evaluate the in vitro effects of drugs related to AR.



## Specifications

<b>Quantity</b>	5E6 Cells per vial, 1 mL
<b>Product Format</b>	1 vial of frozen cells
<b>Shipping</b>	Shipped on dry ice
<b>Storage Conditions</b>	Liquid nitrogen immediately upon receipt

<b>Recovery Medium</b>	DMEM+10% FBS+1% P.S
<b>Growth medium</b>	DMEM+10% FBS+1% P.S+4 µg/mL Blasticidin+0.75 µg/mL Puromycin
<b>Note</b>	None
<b>Freezing Medium</b>	90% FBS+10% DMSO
<b>Growth properties</b>	Adherent
<b>Growth Conditions</b>	37°C, 5% CO <sub>2</sub>

<b>Mycoplasma Testing</b>	The cell line has been screened to confirm the absence of Mycoplasma species.
<b>Safety considerations</b>	Biosafety Level 2
<b>Note</b>	It is recommended to expand the cell culture and store a minimum of 10 vials at an early passage for potential future use.

## Materials

Reagent	Manufacturer/Catalogue No.
DMEM	Gibco/C11995500BT
Fetal Bovine Serum	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/ <a href="#">GM-040404</a>
Puromycin	Genomeditech/ <a href="#">GM-040401</a>
Androgen Receptor(D6F11) Rabbit Monoclonal Antibody	CST/#5153
11-Ketodihydrotestosterone	MCE/HY-135794
Metribolone	GLPBIO/GC19800
Bicalutamide	MCE/HY-14249
Enzalutamide	MCE/HY-70002

## Figures

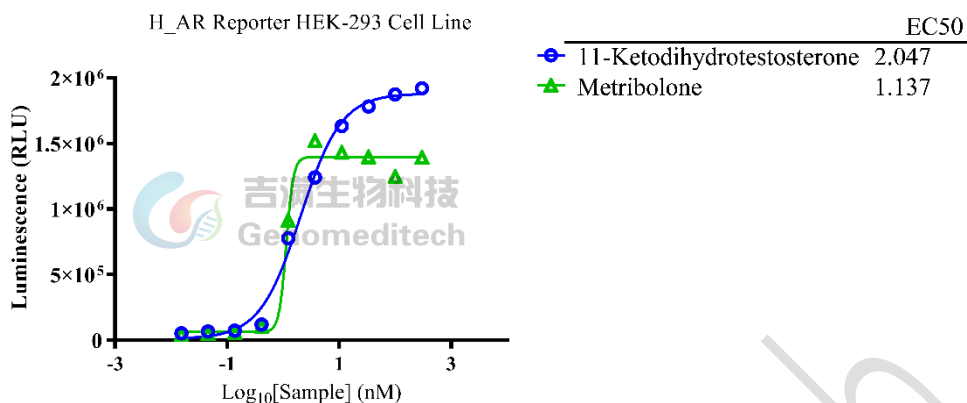


Figure 1 | Response to 11-Ketodihydrotestosterone and Metribolone. The H\_AR Reporter 293 Cell Line (Cat. GM-C45341) at a density of 1.5E4 cells/well (96-well format) were stimulated with serial dilutions of 11-Ketodihydrotestosterone(MCE/HY-135794) and Metribolone (GLPBIO/GC19800) in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [33.8] and [28.0]. Data are shown by drug molar concentration.

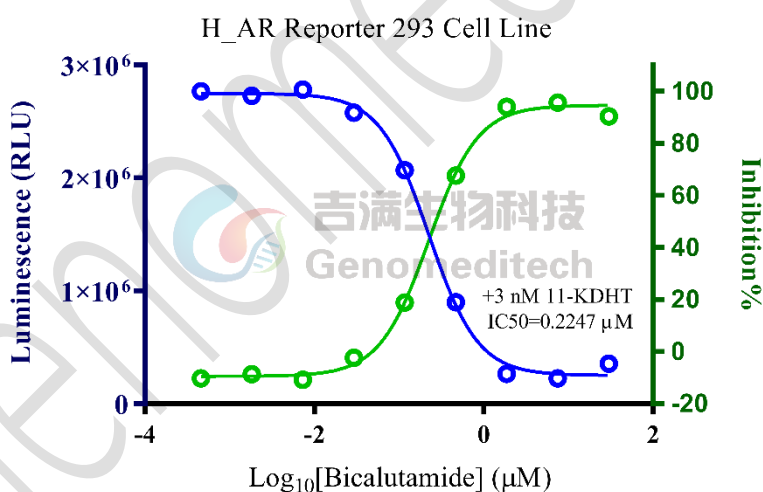


Figure 2 | Inhibition of 11-Ketodihydrotestosterone induced reporter activity by Bicalutamide. Serial dilutions of the Bicalutamide(MCE/HY-14249) was incubated with 1.5E4 cells/well of the H\_AR Reporter 293 Cell Line (Cat. GM-C45341) in a 96-well plate for 1 hour in assay buffer (DMEM +1% FBS+1% P.S). Subsequently, the 11-Ketodihydrotestosterone (MCE/HY-135794) was added to each well at a final concentration of 3 nM,, and the coculture proceeded for an additional 6 hours. Firefly luciferase activity is then measured using the Luciferase Reporter Assay Kit (Genomeditech)(left Y-axis, relative luminescence units), with inhibition percentages shown on the right Y-axis.

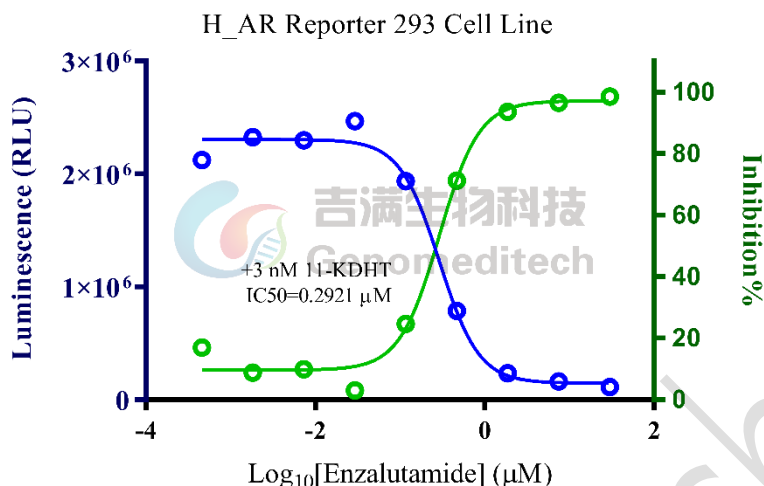


Figure 3 | Inhibition of 11-Ketodihydrotestosterone induced reporter activity by Enzalutamide. Serial dilutions of the Enzalutamide(MCE/HY-70002) was incubated with 1.5E4 cells/well of the H\_AR Reporter 293 Cell Line (Cat. GM-C45341) in a 96-well plate for 1 hour in assay buffer (DMEM +1% FBS+1% P.S). Subsequently, the 11-Ketodihydrotestosterone (MCE/HY-135794) was added to each well at a final concentration of 3 nM,, and the coculture proceeded for an additional 6 hours. Firefly luciferase activity is then measured using the Luciferase Reporter Assay Kit (Genomeditech)(left Y-axis, relative luminescence units), with inhibition percentages shown on the right Y-axis.

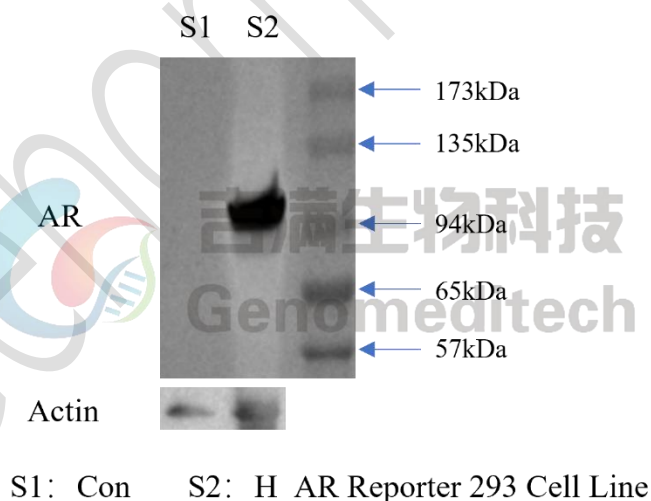


Figure 4 | The protein expression levels of AR in the H\_AR Reporter 293 Cell Line(Cat. GM-C45341) were determined by Western blotting (WB).

## Cell Recovery

Recovery Medium: DMEM+10% FBS+1% P.S

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at  $-70^{\circ}\text{C}$ . Storage at  $-70^{\circ}\text{C}$  will result in loss of viability.

- a) Thaw the vial by gentle agitation in a  $37^{\circ}\text{C}$  water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 - 3 minutes).
- b) Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
- c) Transfer the vial contents to a centrifuge tube containing 5.0 mL complete culture medium and spin at approximately  $176 \times g$  for 5 minutes. Discard supernatant.
- d) Resuspend cell pellet with the recommended recovery medium. And dispense into appropriate culture dishes.
- e) Incubate the culture at  $37^{\circ}\text{C}$  in a suitable incubator. A 5%  $\text{CO}_2$  in air atmosphere is recommended if using the medium described on this product sheet.

## Cell Freezing

Freezing Medium: 90% FBS+10% DMSO

- a) Centrifuge at  $176 \times g$  for 3 minutes to collect cells.
- b) Resuspend the cells in pre-cooled freezing medium and adjust the cell density to  $5 \times 10^6$  cells/mL.
- c) Aliquot 1 mL into each vial.
- d) Place the vial in a controlled-rate freezing container and store at  $-80^{\circ}\text{C}$  for at least 1 day, then transfer to liquid nitrogen as soon as possible.

## Cell passage

Growth medium: DMEM+10% FBS+1% P.S+4  $\mu\text{g/mL}$  Blasticidin+0.75  $\mu\text{g/mL}$  Puromycin

For the first 1 to 2 passages post-resuscitation, use the recovery medium. Once the cells have stabilized, switch to a growth medium.

- a) Subculturing is necessary when the cell density reaches 80%. It is recommended to perform subculturing at a ratio of 1:3 to 1:4 every 2-3 days. Ensure that the density does not exceed 80%, as overcrowding can lead to reduced viability due to compression.
- b) Remove and discard culture medium.
- c) Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
- d) Add 1.0 mL of 0.25% (w/v) Trypsin-EDTA solution to dish and observe cells under an inverted microscope until cell layer is dispersed (usually within 30 to 60 seconds at  $37^{\circ}\text{C}$ ).
- e) Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at  $37^{\circ}\text{C}$  to facilitate dispersal.
- f) Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.
- g) After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.
- h) Incubate cultures at  $37^{\circ}\text{C}$ .

**Subcultivation Ratio: A subcultivation ratio of 1:3 - 1:4 is recommended**

**Medium Renewal: Every 2 to 3 days**

## Notes

- a) Upon initial thawing, a higher number of dead cells is observed, which is a normal phenomenon. Significant improvement is seen after adaptation. Once the cells reach a stable state, the number of dead cells decreases after subculturing and the cell growth rate becomes stable.
- b) Ensure that the cell density does not exceed 80%, as overcrowding may lead to reduced viability due to compression.

## License Agreement:

**By purchasing and using this cell line product, the user voluntarily agrees to accept and abide by the following policies:**

- This cell line product is restricted to research use only and shall not be used for any commercial purposes.
- This product is strictly prohibited from being used in the diagnosis or treatment of human or animal diseases, and shall not be directly used in experiments involving humans.
- Users and their contractors engaged for their benefit may use this material and its derivatives only within the agreed research scope; modification of the material is not permitted, nor may it be distributed, sold, transferred, or otherwise provided to any other entity (including affiliates).
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