Product Datasheet

ARE Luciferase - (LUCPorter™) Stable Reporter Cell Line
NBP2-32795

Unit Size: 1 Vial

Store in gas phase of liquid nitrogen.

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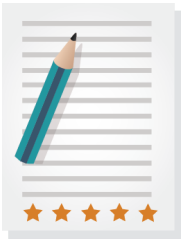

NBP2-32795

ARE Luciferase - (LUCPorter™) Stable Reporter Cell Line

Product Information	
Unit Size	1 Vial
Concentration	Concentration is not relevant for this product. Please see the protocols for proper use of this product.
Storage	Store in gas phase of liquid nitrogen.
Reconstitution Instructions	Complete Growth Medium: DMEM with 4.5 g/L glucose + 10% FBS + 4 mM L-glutamine + 1 mM sodium pyruvate + 100 units/ml penicillin + 0.1 mg/ml streptomycin (Note: The selection agents for this cell line is puromycin at 1 ug/ml).

Product Description	
Description	The ARE/LUCPoter(TM) reporter cell line is designed to monitor the induction of ARE and can be used for screening of agonists, antagonists or signaling inhibitors of ARE induction (Nrf2 activity) as well as for studying the Keap1/Nrf2 signaling pathways. Contents: 3-4 x 10^6 cells Biosafety Level: 1
Host	MCF7
Growth Properties	Adherent Morphology : Epithelial
Selection Agent	Puromycin at 1 ug/ml
Immunogen	The ARE/LUCPorter(TM) reporter cell line is a stably transfected MCF7 cell line which expresses an optimized Renilla luciferase reporter gene (RenSP) under the transcriptional control of the antioxidant response element (ARE). ARE is known to regulate expression and induction of various detoxifying enzyme genes in response to antioxidants and xenobiotics, and is primarily regulated by the Keap1-Nrf2 pathway in which induction and nuclear translocation of Nrf2 mediated by antioxidants and xenobiotics results in the binding of Nrf2 to ARE leading to the expression of defensive genes. One of the antioxidants, curcumin, is known to upregulate Nrf2 leading to activation of the AREs. As also shown in Figure 1, the ARE/ LUCPorter(TM) reporter cell line can be activated by curcumin, in which curcumin activated the cell line in a dose response manner (Figure 1).

Product Application Details	
Applications	Ligand Activation
Recommended Dilutions	Ligand Activation

Procedures

Product Handling Protocol (NBP2-32795)
Note: To ensure the highest cell viability, it is strongly recommended that one should thaw the vial and initiate the cell culture as soon as possible upon receipt. If continued storage of the frozen vial upon receipt is necessary, it should be immediately stored in liquid nitrogen but not at -80C. Storage at -80C will lead to significant loss of cell viability. Please read the entire data sheet before thawing. It is recommended that users follow good tissue culture practice. The reporter line is sterile and all work should be performed under sterile conditions.

1. Prepare a sterile 15-ml tube with 9 ml fresh medium without selection agents pre-warmed at 37C.

2. Thaw the frozen cell vial quickly in a 37C water bath, keeping the cap portion out of the water to avoid any possible contamination.

3. Upon thawing, take the vial out of the water and clean it with 70% ethanol to decontaminate.

4. Transfer contents to the 15-ml tube (Step 1) and mix with medium by gentle inversion of tube.

5. Centrifuge at 1,000 RPM for 5 minutes.

6. Remove supernatant and resuspend cells in 10 ml of fresh medium without selection agents. Note: It is important to grow the cells at this stage without selection agents.

7. Transfer cells into a 25-cm2 tissue culture flask and incubate at 37C in a 95% air-5% CO2 mixture.

8. After cells settle down (in 1-3 days), remove the medium containing minor floating cells and replace with fresh complete growth medium containing selection agents.

9. Whenever the cells are 70-80% confluent, detach the cells by trypsinization and split into new flasks with fresh complete growth medium.

10. Freeze the reporter cell line at 3-4 x 10^6 cells/ml per cryogenic vial. For optimal cell viability after freezing, freeze cells when they have reached log phase growth (95-98% confluency). Detach cells by trypsinization at 37C for 5 min, and harvest cells by mixing with 3 volumes of fresh medium followed by centrifugation (Step 5). Re-suspend the cell pellet in freeze media (FBS with 10% DMSO). Add cell suspension to cryogenic vials in 1 ml aliquots. Place cryogenic vials, in a tissue culture approved cryogenic vial container, in -80C Freezer for 24-48 hours. After 24-48 hours, move the vials into liquid nitrogen storage.

Product Handling Guide (NBP2-32795)
Assume all cultures are hazardous since they may harbor latent viruses or other organisms that are uncharacterized. The following safety precautions should be observed.
Use pipette aids to prevent ingestion and keep aerosols down to a minimum.

No eating, drinking or smoking while handling cells.

Wash hands after handling cultures and before leaving the lab.

Decontaminate work surface with disinfectant or 70% ethanol before and after working with cells.

All waste should be considered hazardous.

Dispose of all liquid waste after each experiment and treat with bleach.

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Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Reporter Cell Lines are guaranteed for 1 year from date of receipt.

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