

# Product Datasheet

## iNOS Luciferase - (LUCPorter™) Stable Reporter Cell Line NBP2-32782

Unit Size: 1 Vial

Store in gas phase of liquid nitrogen.

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**NBP2-32782****iNOS Luciferase - (LUCPorter™) Stable Reporter Cell Line**

<b>Product Information</b>	
<b>Unit Size</b>	1 Vial
<b>Concentration</b>	Concentration is not relevant for this product. Please see the protocols for proper use of this product.
<b>Storage</b>	Store in gas phase of liquid nitrogen.
<b>Reconstitution Instructions</b>	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 3 ug/ml).

<b>Product Description</b>	
<b>Description</b>	<p>The iNOS/LUCPorter(TM) reporter cell line is designed to monitor the induction of iNOS and can be used for screening of agonists, antagonists or signaling inhibitors of iNOS induction as well as for studying the iNOS induction-related signaling pathways.</p> <p>Contents: 3-4 x 10<sup>6</sup> cells Biosafety Level 2</p>
<b>Host</b>	RAW264.7
<b>Gene ID</b>	4843
<b>Gene Symbol</b>	NOS2
<b>Growth Properties</b>	Adherent Morphology : Macrophage
<b>Selection Agent</b>	Puromycin at 3 ug/ml
<b>Immunogen</b>	iNOS Luciferase - (LUCPorter (TM)) Stable Reporter Cell Line is a stably transfected RAW 264.7 cell line which expresses an optimized Renilla luciferase reporter gene (RenSP) under the transcriptional control of the iNOS promoter. Inducible nitric oxide synthase (iNOS) is an inducible enzyme that catalyzes the production of nitric oxide (NO) from L-arginine. the pathogenesis of septic shock. NO is one of the smallest signaling molecules that can diffuse into the cell and is involved in various physiological functions, pathogenesis of septic shock, many diseases associated with autoimmunity, and tumorigenesis. iNOS gene is generally known to be induced by various proinflammatory cytokines and pathogen-associated molecular patterns such as TLR ligands. As shown in Figure 1, the iNOS promoter activity in the iNOS/LUCPorter(TM) reporter cell line was induced by various TLR ligands (Figure 1).

<b>Product Application Details</b>	
<b>Applications</b>	Ligand Activation
<b>Recommended Dilutions</b>	Ligand Activation
<b>Application Notes</b>	<p>Ligand activation: See image for Induction of iNOS promoter activity by various TLR ligands and phorbol 12-myristate 13-acetate (PMA). The iNOS/LUCPorter (TM) RAW cell line was plated in 96-well white plates at 8.5 x 10<sup>5</sup> cells/well. After 16 h, cells were stimulated with 10 ng/ml Pam3CSK4 (TLR1/2 ligand; NBP2-25297), 10 ng/ml MALP-2 (TLR2/6 ligand; NBP2--26219), 40 ug/ml Poly(I:C) (TLR3 ligand; NBP2-25288), 0.5 ug/ml LPS (TLR4 ligand; NBP2-25295), 100 ng/ml Flagellin (TLR5 ligand; NBP2-25289), 10 ug/ml R848 (TLR7/8 ligand; NBP2-26231), 10 ug/ml mCpG (mouse TLR9 ligand; NBP2-26235) and 100 ng/ml PMA for 16 h. Luciferase activity was then analyzed by directly adding the complete mixture of luciferase reporter assay reagent (NBP2-25287) into each well of the plate. After 10 min, the plate was read in a plate luminometer.</p>





## Procedures

### Product Handling Protocol (NBP2-32782)

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-cm<sup>2</sup> tissue culture flask and incubate at 37C in a 95% air-5% CO<sub>2</sub> 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  $3-4 \times 10^6$  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 (NBP2-32782)

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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