

# Product Datasheet

## Human TLR4/NF- $\kappa$ B NF- $\kappa$ B/SEAP - (SEAPorter™) Stable Reporter Cell Line NBP2-26276

Unit Size: 1 Vial

Store in gas phase of liquid nitrogen.

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**NBP2-26276****Human TLR4/NF-kB NF-kB/SEAP - (SEAPorter™) 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 + 10 ug/ml blasticidin + 0.5 mg/ml G418 (Geneticin).
<b>Product Description</b>	
<b>Description</b>	The TLR4 reporter cell line is a stably co-transfected cell line which expresses full-length human Toll-like receptor 4 (TLR4) and the secreted alkaline phosphatase (SEAP) reporter gene under the transcriptional control of an NF-kB response element.
<b>Host</b>	HEK293
<b>Target Species</b>	Human
<b>Reporter Gene</b>	Secreted alkaline phosphatase (SEAP)
<b>Growth Properties</b>	Adherent Morphology: Epithelial
<b>Selection Agent</b>	Blasticidin and G418.
<b>Specificity/Sensitivity</b>	TLR4/NF-kB/SEAP
<b>Notes</b>	<p>Assume all cultures are hazardous since they may harbor latent viruses or other organisms that are uncharacterized. The following safety precautions should be observed.</p> <ul style="list-style-type: none"> <li>- Use pipette aids to prevent ingestion and keep aerosols down to a minimum.</li> <li>- No eating, drinking or smoking while handling the TLR4 reporter line.</li> <li>- Wash hands after handling the TLR4 reporter line and before leaving the lab.</li> <li>- Decontaminate work surface with disinfectant or 70% ethanol before and after working with cells.</li> <li>- All waste should be considered hazardous.</li> <li>- Dispose of all liquid waste after each experiment and treat with bleach.</li> </ul>
<b>Lysate Type</b>	Cell
<b>Product Application Details</b>	
<b>Applications</b>	Flow Cytometry, Ligand Activation
<b>Recommended Dilutions</b>	Flow Cytometry, Ligand Activation
<b>Application Notes</b>	<p>The TLR4 reporter line can be used for TLR4-dependent functional assays as well as screening of TLR4 agonists or antagonists. The TLR4 reporter cell line has been validated by flow cytometry (Fig. 1). Functional activity of this cell line has been validated by transfecting the MD2 plasmid and analyzing SEAP activity with SEAPorter™ Assay Kit (NBP2-25285) (Fig. 2).</p> <p>Contents: 3~4 x 10<sup>6</sup> cells Biosafety Level: 2</p>

## Publications

Lee Y, Lee IY, Yun HJ et al. BAT3 negatively regulates lipopolysaccharide-induced NF- $\kappa$ B signaling through TRAF6 Biochem. Biophys. Res. Commun. 2016-09-16 [PMID: 27501752] (WB, Rat)

O'Neill S, Humphries D, Tse G et al. Heat shock protein 90 inhibition abrogates TLR4-mediated NF- $\kappa$ B activity and reduces renal ischemia-reperfusion injury. Sci Rep. 2015-08-07 [PMID: 26248657] (Func)

Greene CM, Carroll TP, Smith SG et al. TLR-induced inflammation in cystic fibrosis and non-cystic fibrosis airway epithelial cells. J Immunol. 2005-02-01 [PMID: 15661927] (Func)

### Details:

Functional Assays: 1.TLR2/NF- $\kappa$ B/SEAP Stably Transfected HEK 293 Cells (IML-102). 2. TLR4/MD-2/NF- $\kappa$ B/SEAP Stably Transfected HEK 293 Cells (IML-104MD2). 3. TLR4/NF- $\kappa$ B/SEAP Stably Transfected HEK 293 Cells (IML-104).4.TLR7/NF- $\kappa$ B/SEAP Stably Transfected HEK 293 Cells (IML-107). 5. LPS from E. Coli, TLR4 Ligand (IMG-2204). 6. SEAPorter Assay Kit (10055K).

Chandrakesan P, Ahmed I, Anwar T et al. Novel changes in NF- $\kappa$ B activity during progression and regression phases of hyperplasia: role of MEK, ERK, and p38. J Biol Chem. 2010-10-22 [PMID: 20710027]

Chan M, Hayashi T, Mathewson RD et al. Identification of substituted pyrimido[5,4-b]indoles as selective Toll-like receptor 4 ligands. J Med Chem. 2013-06-13 [PMID: 23656327]

Ahmed I, Roy B, Chandrakesan P et al. Evidence of functional cross talk between the Notch and NF- $\kappa$ B pathways in nonneoplastic hyperproliferating colonic epithelium. Am J Physiol Gastrointest Liver Physiol. 2013-02-15 [PMID: 23203159]

### Details:

1. TLR4/NF- $\kappa$ B/SEAPorter HEK 293 cell line (IML-104).

Feng D, Sangster-Guity N, Stone R et al. Differential requirement of histone acetylase and deacetylase activities for IRF5-mediated proinflammatory cytokine expression. J Immunol. 2010-11-15 [PMID: 20935208]



## Procedures

### Product Handling Protocol (NBP2-26276)

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 TLR4 reporter cell 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 TLR4 reporter cell line 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 pellet in 10 ml of fresh medium without selection agents. Note: It is important to grow the reporter cells at this stage without any selection agents.
7. Transfer the TLR4 cell line 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 and replace with fresh complete growth medium containing selection agents.
9. At 70-80% confluency, detach the cells by trypsinization and split into new flasks with fresh complete growth medium.
10. Freeze the TLR4 reporter cell line at  $3\sim 4 \times 10^6$  cells/ml per cryogenic vial. For optimal viability after freezing, freeze cells when they have reached log phase growth (95-98% confluency). Detach by trypsinization at 37C for 5 min, and harvest by mixing with 3 volumes of fresh medium followed by centrifugation (Step 5). Resuspend the pellet in freeze media (FBS with 10% DMSO). Add 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.

### MSDS (NBP2-26276)

#### IDENTIFICATION

Product Name HEK 293 cells (human embryonic kidney), HeLa cells (human epithelial carcinoma) or RAW cells (mouse macrophage) stably transfected; Dimethyl sulfoxide  
 Synonyms Methyl sulfoxide; DMSO; Sulfinylbis (methane)

#### COMPOSITION, INFORMATION ON INGREDIENTS

CAS# none  
 Name Cells, human origin  
 CAS# 67-68-5  
 Chemical Name Dimethyl Sulfoxide  
 Percent 10

#### HAZARDS IDENTIFICATION

##### EMERGENCY OVERVIEW

Appearance: clear liquid. May be absorbed through intact skin. Hygroscopic (absorbs moisture from the air). May cause liver and kidney damage. CAUTION! Causes eye and skin irritation. Causes respiratory tract irritation.  
 Target Organs: Kidneys, liver, eyes, skin, mucous membranes.

#### Potential Health Effects

Eye: Produces irritation, characterized by a burning sensation, redness, tearing, inflammation, and possible corneal



injury. May cause chemical conjunctivitis.

Skin: May cause irritation with burning pain, itching and redness. Substance is rapidly absorbed through the skin.

Ingestion: May cause gastrointestinal irritation with nausea, vomiting and diarrhea. May cause liver and kidney damage. May cause garlic smell on the breath and body.

Inhalation: May cause respiratory tract irritation. Can produce delayed pulmonary edema.

Chronic: Prolonged or repeated skin contact may cause dermatitis. May cause liver and kidney damage. Effects may be delayed.

## FIRST AID MEASURES

Eyes: Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid.

Skin: Get medical aid. Flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse.

Ingestion: Never give anything by mouth to an unconscious person. Get medical aid. Do NOT induce vomiting. If conscious and alert, rinse mouth and drink 2-4 cupfuls of milk or water.

Product Name HEK 293 cells (human embryonic kidney), HeLa cells (human epithelial carcinoma) or RAW cells (mouse

macrophage) stably transfected; Dimethyl sulfoxide

Synonyms Methyl sulfoxide; DMSO; Sulfinylbis (methane)

Inhalation: Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid. Do NOT use mouth-to-mouth resuscitation.

Notes to Physician: Treat symptomatically and supportively.

## FIRE FIGHTING MEASURES

General Information: As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. Vapors may form an explosive mixture with air. During a fire, irritating and highly toxic gases may be generated by thermal decomposition or combustion. Use water spray to keep fire-exposed containers cool. Vapors may be heavier than air. They can spread along the ground and collect in low or confined areas. Containers may explode when heated.

Extinguishing Media: Cool containers with flooding quantities of water until well after fire is out. Use water spray, dry chemical, carbon dioxide, or appropriate foam.

Flash Point: 95 deg C ( 203.00 deg F)

Autoignition Temperature: 215 deg C ( 419.00 deg F)

Explosion Limits, Lower: 2.6 vol %

Upper: 42 vol %

NFPA Rating: (estimated) Health: 1; Flammability: 1; Instability: 0

## ACCIDENTAL RELEASE MEASURES

General Information: Use proper personal protective equipment as indicated in Section 8.

Spills/Leaks: Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container. Do not flush into a sewer. Clean up spills immediately, observing precautions in the Protective Equipment section. Provide ventilation.

## HANDLING AND STORAGE

Handling: Avoid contact with eyes, skin, and clothing. Keep container tightly closed. Avoid ingestion and inhalation. Use with adequate ventilation. Wash clothing before reuse.

Storage: Keep away from heat, sparks, and flame. Store in a tightly closed container. Keep from contact with oxidizing materials. Store in a cool, dry, well-ventilated area away from incompatible substances.

## EXPOSURE CONTROLS, PERSONAL PROTECTION

Engineering Controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate ventilation to keep airborne concentrations low.

Exposure Limits

Chemical Name Dimethyl Sulfoxide

ACGIH None listed

NIOSH None listed

OSHA - Final PELs None listed



OSHA Vacated PELs: Dimethyl sulfoxide: No OSHA Vacated PELs are listed for this chemical.

Personal Protective Equipment Eyes: Wear chemical goggles.

Skin: Wear appropriate protective gloves to prevent skin exposure.

Clothing: Wear appropriate protective clothing to prevent skin exposure.

Respirators: A respiratory protection program that meets OSHA's 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant a respirator's use.

## PHYSICAL AND CHEMICAL PROPERTIES

Physical State: Liquid

Appearance: clear

Odor: slight odor - sulfurous odor - garlic-like odor

pH: Not available.

Vapor Pressure: 0.4 mm Hg at 20 Vapor Density: 2.7 (air=1) Evaporation Rate: Not available. Viscosity: 1.1cp @ 27 deg C Boiling Point: 189 deg C Freezing/Melting Point: 18.4 deg C

Decomposition Temperature: > 200 deg C

Solubility: Soluble.

Specific Gravity/Density: 1.1010g/cm<sup>3</sup> Molecular Formula: C<sub>2</sub>H<sub>6</sub>OS Molecular Weight: 78.13

## STABILITY AND REACTIVITY

Chemical Stability: Stable at room temperature in closed containers under normal storage and handling conditions.

Conditions to Avoid: Excess heat.

Incompatibilities with Other Materials: Strong oxidizing agents, strong acids, strong bases. Hazardous Decomposition

Products: Carbon monoxide, oxides of sulfur, carbon dioxide. Hazardous Polymerization: Has not been reported.

## TOXICOLOGICAL INFORMATION RTECS#

CAS# 67-68-5: PV6210000

### LD50/LC50

CAS# 67-68-5:

Draize test, rabbit, eye: 100 mg;

Draize test, rabbit, eye: 500 mg/24H Mild; Draize test, rabbit, skin: 500 mg/24H Mild; Oral, mouse: LD50 = 7920 mg/kg;

Oral, rat: LD50 = 14500 mg/kg; Skin, rat: LD50 = 40 gm/kg;<BR.

### Carcinogenicity

CAS# 67-68-5: Not listed by ACGIH, IARC, NIOSH, NTP, or OSHA.

Epidemiology: No information available. Teratogenicity: No information available. Reproductive Effects: No information available. Neurotoxicity: No information available.

Mutagenicity: No information available.

Other Studies: See actual entry in RTECS for complete information.

## ECOLOGICAL INFORMATION

Ecotoxicity: No data available. No information available.

Environmental: Terrestrial: Expected to be mobile in soil, due to its high water solubility. Some volatilization from dry soil and surfaces may be expected. Aquatic: Dimethyl sulfoxide disproportionates in water to dimethyl sulfide and dimethyl sulfone, a reaction catalyzed by light. Atmospheric: Exists primarily in the vapor phase and be removed by both wet and dry deposition. It will react with photochemically-produced hydroxyl radicals with a half-life of about 7 hr. DMSO is very difficult to biodegrade. Physical: No information available.

Other: For more information, see "HANDBOOK OF ENVIRONMENTAL FATE AND EXPOSURE DATA."

## DISPOSAL CONSIDERATIONS

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.

RCRA P-Series: None listed

RCRA U-Series: None listed



#### SPECIAL PRECAUTIONS

Store at 2-8 degrees C in well-sealed container. Store away from strong oxidizing agents. This product is intended for research use only.

#### DISCLAIMER

For R&D use only. Not for drug, household or other uses.

#### WARRANTY

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. NOVUS, shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale. Copyright 2008 NOVUS License granted to make unlimited paper copies for internal use only.







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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.

For more information on our 100% guarantee, please visit [www.novusbio.com/guarantee](http://www.novusbio.com/guarantee)

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