

Product Datasheet

Mouse NFkB Secreted Alkaline Phosphatase Reporter SEAP - (SEAPorter™) Stable Reporter Cell Line NBP2-26261

Unit Size: 1 Vial

Store in gas phase of liquid nitrogen.

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NBP2-26261**Mouse NFkB Secreted Alkaline Phosphatase Reporter SEAP - (SEAPorter™) 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 + 0.5 mg/ml G418 (Geneticin).

Product Description	
Description	<p>The RAW reporter cell line can be used for screening of TLR agonists or antagonists as well as inhibitory TLR antibody assay. RAW 264.7 cells are known to respond to most Toll-like receptor (TLR) ligands, which trigger the NF-κB induction and lead to inflammatory cytokine production. RT-PCR tests also shows that RAW 264.7 cells produce all of the TLR mRNAs except for TLR5 (Figure 1). Using a 96-well plate format assay, the RAW cell line has been validated by TLR ligand stimulation in which all of the TLR ligands except for TLR5 ligand activated the cell line (Figure 2).</p> <p>Contents: 3~4 x 10⁶ cells Biosafety Level: 2</p>
Host	RAW264.7
Target Species	Mouse
Reporter Gene	Secreted alkaline phosphatase (SEAP)
Growth Properties	Adherent Morphology: Macrophage
Selection Agent	RAW cell line.
Specificity/Sensitivity	RAW NF- κ B/SEAP Reporter Cell Line
Immunogen	The RAW reporter stable cell line is a stably transfected RAW 264.7 cell line which expresses the secreted alkaline phosphatase (SEAP) reporter gene under the transcriptional control of an NF- κ B response element.
Notes	<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"> - Use pipette aids to prevent ingestion and keep aerosols down to a minimum. - No eating, drinking or smoking while handling the RAW line. - Wash hands after handling the RAW line and before leaving the lab. - Decontaminate work surface with disinfectant or 70% ethanol before and after working with stable cells. - All waste should be considered hazardous. - Dispose of all liquid waste after each experiment and treat with bleach.
Lysate Type	Cell

Product Application Details	
Applications	In vitro assay, Ligand Activation
Recommended Dilutions	In vitro assay, Ligand Activation

Publications

Zarpelon AC, Fattori V, Souto FO et al. The Sesquiterpene Lactone, Budlein A, Inhibits Antigen-Induced Arthritis in Mice: Role of NF- κ B and Cytokines Inflammation 2017-08-05 [PMID: 28780730] (In vitro)

Park Jh, Jun Jg, Kim Jk. (E)-3-(3,4-dihydroxy-2-methoxyphenyl)-1-(2,4-dihydroxyphenyl)prop-2-en-1-one, a novel licochalcone B derivative compound, suppresses lipopolysaccharide-stimulated inflammatory reactions in RAW264.7 cells and endotoxin shock in mice Chemico-Biological Interactions et al. 2014-12-05 [PMID: 25451593] (In vitro)

Details:

Mouse nF κ B Secreted Alkaline Phosphatase Reporter SEAP - (SEAPorter(TM)) Stable Reporter Cell Line (also called RAW nF- κ B/SEAP Reporter Cell Line) used for nF- κ B reporter assay - cells were pretreated or not with (E)-3-(3,4-dihydroxy-2-methoxyphenyl)-1-(2,4-dihydroxyphenyl)prop-2-en-1-one, a novel licochalcone B derivative compound and then were stimulated with 500 ng/ml LPS for 24 h (Fig. 4A)

Potzsch S, Blankenhorn A, Navarrete Santos A et al. The effect of an AGE-rich dietary extract on the activation of NF- κ B depends on the cell model used. Food Funct. 2013-07-01 [PMID: 23426622]

Details:

IMGENEX products cited: 1. RAW NF- κ B/SEAP Reporter Cell Line (IML-120): Figs 3, 4 2. SEAPorter Assay Kit (10055K): Fig 3 Note: Bread crumb extract (BCE) was used as an advanced glycation end product to treat RAW IML-120 cells. NF- κ B activation was measure

Namachivayam K, Blanco CL, Frost BL et al. Preterm human milk contains a large pool of latent TGF- β , which can be activated by exogenous neuraminidase. Am J Physiol Gastrointest Liver Physiol. 2013-06-15 [PMID: 23558011]

Details:

Products cited (Fig 5C): 1. RAW NF- κ B/SEAP Reporter Cell Line (IML-120) 2. SEAPorter Assay Kit (10055K) Note: The results showed LPS activation of SEAP activity in the IML-120 cells could be attenuated by pre-treating the cells with pre-term milk + neuram

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 (NBP2-26261)

Please read the entire data sheet before thawing. It is recommended that users follow good tissue culture practice. The RAW 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 RAW 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. It is important to grow the RAW cell line at this stage without any selection agents.
7. Transfer the RAW line 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 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 RAW cell line at $3\text{--}4 \times 10^6$ 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 (NBP2-26261)

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/cm³ Molecular Formula: C₂H₆OS 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

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