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.

Publications: 5
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Updated 10/10/2018 v.20.1

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**Product Information**

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<th><strong>Unit Size</strong></th>
<th>1 Vial</th>
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<tr>
<td><strong>Concentration</strong></td>
<td>Concentration is not relevant for this product. Please see the protocols for proper use of this product.</td>
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<tr>
<td><strong>Storage</strong></td>
<td>Store in gas phase of liquid nitrogen.</td>
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<td><strong>Reconstitution Instructions</strong></td>
<td>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).</td>
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**Product Description**

**Description**

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-kB 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).

Contents: 3~4 x 10^6 cells 
Biosafety Level: 2

**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-kB/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-kB response element.

**Notes**

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 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
In vitro assay: Mouse NFkB Secreted Alkaline Phosphatase SEAP - (SEAPorter™) Stable Reporter Cell Line [NBP2-26261] - mRNA expression patterns of Toll-like receptors in RAW264.7 cells. Total RNAs were prepared and reverse transcription (RT) was performed to produce cDNAs. PCR was done using the gene-specific primers for mouse TLR1 to TLR9, MD2 as well as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a house-keeping gene control.

Ligand Activation: Mouse NFkB Secreted Alkaline Phosphatase SEAP - (SEAPorter™) Stable Reporter Cell Line [NBP2-26261] - TLR ligand stimulation assay. The RAW cell line was plated in 96-well plates at 5 x 10^4 cells/well. After 16 h, cells were stimulated with MALP-2, Pam3CSK4, Poly(I:C), LPS, Flagellin, R848 or mCpG as noted in each graph for 24 h. SEAP was analyzed using SEAPorter™ Assay Kit.
<table>
<thead>
<tr>
<th>Publications</th>
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<tbody>
<tr>
<td><strong>Zarpelon AC, Fattori V, Souto FO et al.</strong> The Sesquiterpene Lactone, Budlein A, Inhibits Antigen-Induced Arthritis in Mice: Role of NF-kB and Cytokines Inflammation 2017 Aug 05 [PMID: 28780730] (In vitro)</td>
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<tr>
<td><strong>Park Jh, Jun Jg, Kim Jk.</strong> (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 Dec 05 [PMID: 25451593] (In vitro)</td>
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<tr>
<td><strong>Details:</strong> Mouse nFkB Secreted Alkaline Phosphatase Reporter SEAP - (SEAPorter(TM)) Stable Reporter Cell Line (also called RAW nF-kB/SEAP Reporter Cell Line ) used for nF-kB 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)</td>
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<td><strong>Potzsch S, Blankenhorn A, Navarrete Santos A et al.</strong> The effect of an AGE-rich dietary extract on the activation of NF-kB depends on the cell model used. Food Funct. 2013 Jul [PMID: 23426622]</td>
</tr>
<tr>
<td><strong>Details:</strong> IMGENEX products cited: 1. RAW NF-kB/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-kB activation was measure</td>
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<td><strong>Namachivayam K, Blanco CL, Frost BL et al.</strong> Preterm human milk contains a large pool of latent TGF-B, which can be activated by exogenous neuraminidase. Am J Physiol Gastrointest Liver Physiol. 2013 Jun 15 [PMID: 23558011]</td>
</tr>
<tr>
<td><strong>Details:</strong> Products cited (Fig 3C): 1. RAW NF-kB/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</td>
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<td><strong>Details:</strong> TLR9 Stably Transfected HEk 293 cells (IML-209). FA: TLR9 expressing HEk293 cells expressing TLR9 were transfected with the IL6 luciferase promoter reporter then stimulated with CpG-B, TSA, or both, Fig S1B.</td>
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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 37°C.

2. Thaw the RAW cell line vial quickly in a 37°C 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 37°C 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 3~4 x 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 37°C 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 -80°C 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 deg C
Vapor Density: 2.7 (air=1)
Evaporation Rate: Not available.
Viscosity: 1.1 cp @ 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: C₂H₆O₅S
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;

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