

NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection System

Catalog nos. N10559, N10560

Table 1. Contents and storage information.

Material	Composition	N10559	N10560	Storage	Stability
NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection Kit 2–6°C Components					
Assay buffer (Component A)	Contains a proprietary mixture of non-placental alkaline phosphatase inhibitors	20 mL	60 mL	<ul style="list-style-type: none">• 2–6°C• Protect from light• DO NOT FREEZE	When stored as directed, this kit is stable for 1 year.
Reaction buffer (Component B)	Contains Emerald™ luminescence enhancer	19 mL	57 mL		
5X dilution buffer (Component C)	–	5 mL	15 mL		
CSPD® substrate (Component D)	–	1 mL	3 mL		
NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection Kit ≤–20°C Components					
Control enzyme	0.3 ng/μL purified human placental alkaline phosphatase in 150 mM of Tris (pH 7.8), 50 mM NaCl, 50% glycerol	50 μL	50 μL	<ul style="list-style-type: none">• ≤–20°C	When stored as directed, this kit is stable for 1 year.
Number of assays: Sufficient material is supplied for 400 (Cat. no. N10559) or 1,200 (Cat. no. N10560) microplate assays based on the protocol below.					

Introduction

The NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection System is a chemiluminescent reporter gene assay system designed for the rapid and sensitive detection of secreted placental alkaline phosphatase (SEAP) in cell culture media. SEAP is a reporter protein that is secreted into the cell culture media and detected by testing aliquots of media, leaving cells intact for further experimentation.^{1,2} SEAP is a truncated form of human placental alkaline phosphatase (PLAP). Detection of non-secreted placental alkaline phosphatase is also possible (see Preparing Extracts for Non-Secreted Placental Alkaline Phosphatase and Direct Lysis Procedure for Microplate Cultures).

Description of the System

The NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection System incorporates CSPD® chemiluminescent substrate and Emerald™ luminescence enhancer for high sensitivity and wide dynamic range.^{3,4} The system has been used for detection of secreted placental alkaline phosphatase reporter enzyme in cell culture media^{5,6} and for quantitation of non-secreted placental alkaline phosphatase in both cell and tissue extracts.^{7,8}

The NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection assay is simple and rapid. Secreted placental alkaline phosphatase is measured from 48 to 72 hours after cell transfection.² Cell culture medium or cell lysate is incubated first with a buffer system that differentially inhibits non-placental alkaline phosphatase (serum and endogenous cellular alkaline phosphatase) and then with CSPD®-containing reaction buffer until maximum light emission is reached (approximately 20 minutes). The light emission kinetics provide a persistent glow signal that enables measurement over a wide time interval. Light signal output is measured in a luminometer, without the need for automated injection capability.

Chemiluminescent reporter assays for secreted placental alkaline phosphatase may be conducted in cells that have endogenous non-placental alkaline phosphatase activity. Endogenous non-placental enzyme activity is significantly reduced with a combination of heat inactivation and differential inhibitors that do not significantly inhibit the transfected placental alkaline phosphatase. It is important to determine the level of endogenous enzyme in media of non-transfected cells in order to establish assay background. Certain cell lines, such as HeLa and others derived from cervical cancers, may express placental alkaline phosphatase which may produce high assay backgrounds when shed into the media.⁹ Therefore, we do **not** recommend the use of secreted alkaline phosphatase as a reporter system in these cell lines.

Applications

The NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection System has been widely used for the following applications:

- Reporter gene assays measuring gene expression in established cell lines¹⁰ and in transfected primary cells,^{11,12} including as a gene knockdown/RNA interference read-out.¹³
- Viral functional assays, including viral gene expression assays,^{14,15} viral replication,^{16,17} viral fusogenicity,¹⁸ virus neutralization and viral-mediated cell-cell fusion,¹⁹ and viral infectivity.²⁰
- For assaying serum samples from transgenic, transfected, or viral vector-infected animals in *in vivo* reporter gene assays to measure SEAP levels in mouse,²¹ rat,²² marmoset,²³ monkey²⁴ and pig sera,²⁵ and in chicken egg allantoic fluid.²⁶
- For the sensitive detection of mouse SEAP protein (mSEAP),²¹ which has been developed for improved SEAP protein stability in transgenic mice.
- For measuring SEAP as a functional reporter for receptor-ligand binding assays with a SEAP-ligand chimera,²⁷ protease-mediated secretion,²⁸ and for secretion pathway activity,^{29,30} including as a functional assay to measure effects of siRNA-mediated protein knockdown on specific protein secretion pathways.³¹
- For measuring non-placental alkaline phosphatase as a biomarker.¹⁰

Before Starting

Materials Required but Not Provided

- Mammalian cells in adherent or suspension culture and culture medium
- SEAP reported expression vector
- Phosphate buffered saline (PBS)
- Triton® X-100
- Deionized water
- 96-well tissue culture-treated luminometer microplates
- Microplate luminometer

Caution

Component A contains diethanolamine and is harmful. Prolonged exposure to Component A can cause serious damage to health. It is irritating to skin and presents risk of serious damage to eyes. It is harmful to aquatic organisms, and may cause long-term adverse effects in the aquatic environment. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. If swallowed, seek medical advice immediately.

Components B and D are irritating to eyes and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and eye/face protection.

General Guidelines

- Read the entire Experimental Protocols section before proceeding. Perform all assays in triplicate at room temperature, unless otherwise indicated.
- The NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection System assay system is **not** provided with SEAP reporter expression vectors. These are available through various commercial suppliers for cell culture transfection as well as *in vivo* delivery and expression.
- Adding assay buffer (Component A) to warm culture media or heating culture media with assay buffer may result in decreased sensitivity due to increased background. Eliminating or decreasing the incubation time in assay buffer may have the same effect, since non-placental alkaline phosphatase (PLAP) background activity will not be inhibited to the same extent.
- We recommend using a single-mode luminometer or a multi-mode detection instrument set for luminescence measurement to measure light emission from 96- or 384-well microplates. Optimize the number of cells used per well to prevent a measurement signal that is outside the linear range of the luminometer. Extremely high light signals can saturate the detector (very unlikely for experimental samples), resulting in erroneous measurements. Refer to your luminometer user's manual to determine the upper limit for your specific luminometer.
- To perform the detection assay on the maximum number of samples (see **Number of Assays** in Table 1), use the smaller volume of 1X dilution buffer specified in the protocols below, and follow the rest of the protocol as indicated (*e.g.*, use 50 µL of reagent if the range given is 50–150 µL). If using the smaller volume of 1X dilution buffer, samples will be more concentrated than if the maximum volume indicated is used. Therefore, it may be possible or more ideal to use a smaller volume of the original culture medium sample.

Controls Recommended positive and negative controls are listed below.

Positive Control

The control enzyme supplied with the kit, purified human placental alkaline phosphatase, provides a positive control for the assay reagents, as well as a means to determine the range of detection of the luminometer instrumentation, if desired. The purified enzyme standard curve is not intended (or accurate) for absolute quantitation of reporter enzyme concentrations, as the specific activity of the purified enzyme preparation and the reporter enzyme may differ significantly. Additional positive controls can include use of control SEAP constructs that provide constitutive expression of reporter enzyme as a positive control for cell transfection.

The stock control enzyme is approximately 0.3 ng/ μ L (0.75 U/mL) in 150 mM Tris (pH 7.8), 50 mM NaCl, 50% glycerol. Generate a standard curve by serially diluting the stock enzyme in 1X dilution buffer or mock-transfected cell culture media. Use a 10 μ L aliquot of the stock enzyme (undiluted) for the high end detection limit.

Alternatively, you can prepare stock enzyme by reconstituting lyophilized human placental alkaline phosphatase (Sigma Cat. no. P-3895) to 1 mg/mL in 1X dilution buffer containing 0.1% BSA and 50% glycerol. Store at -20°C .

Negative Control

Assay a volume of mock-transfected extract equivalent to that of experimental extract to determine endogenous cellular background. In experiments involving induction of reporter expression, assay uninduced cells as a negative control for total assay background.

Experimental Protocols

**Detection with
Tube Luminometers**

This procedure is for detecting secreted placental alkaline phosphatase (SEAP) in 100 μ L of culture medium using a tube luminometer.

For preparing lysates for detection of non-secreted placental alkaline phosphatase expression, see **Preparing Extract for Non-Secreted Placental Alkaline Phosphatase and Direct Lysis Procedure for Microplate Cultures**.

- 1.1 To prepare working reaction buffer (100 μ L/tube), dilute sufficient CSPD[®] substrate (Component D) 1:20 in reaction buffer (Component B).
- 1.2 Equilibrate assay buffer (Component A) (100 μ L/tube) and working reaction buffer (prepared in step 1.1) to room temperature.
- 1.3 Dilute sufficient 5X dilution buffer (Component C) 1:5 in deionized water to obtain 100–300 μ L of 1X dilution buffer per sample.
- 1.4 Prepare a sample by diluting 100 μ L of culture medium with 100–300 μ L of 1X dilution buffer (prepared in step 1.3) in a microcentrifuge tube.

Note: To perform the detection assay on the maximum number of samples, use 100 μ L of the 1X dilution buffer, and follow rest of protocol as indicated (see **General Guidelines**).

- 1.5 Heat the diluted sample (prepared in step 1.4) at 65°C for 30 minutes, then cool on ice to room temperature.
- 1.6 Add 100 μ L of diluted sample (from step 1.5) to a luminometer tube.

- 1.7 Add 100 μ L of assay buffer (from step 1.2) per tube, and incubate for 5 minutes at room temperature.
- 1.8 Add 100 μ L of working reaction buffer (from step 1.2) per tube, and incubate for 20 minutes at room temperature.
- 1.9 Place tubes in a luminometer and measure chemiluminescence for 0.1–1 second per tube.

Detection with Microplate Luminometers

This procedure is for detecting secreted placental alkaline phosphatase (SEAP) in 100 μ L of culture medium using a tube luminometer.

For preparing lysates for detection of non-secreted placental alkaline phosphatase expression, see **Preparing Extract for Non-Secreted Placental Alkaline Phosphatase** and **Direct Lysis Procedure for Microplate Cultures**.

- 2.1 To prepare working reaction buffer (50 μ L/well), dilute sufficient CSPD® substrate (Component D) 1:20 in reaction buffer (Component B).
- 2.2 Equilibrate assay buffer (Component A) (50 μ L/well) and working reaction buffer (prepared in step 2.1) to room temperature.
- 2.3 Dilute sufficient 5X dilution buffer (Component C) 1:5 in deionized water to obtain 50–150 μ L of 1X dilution buffer per sample.
- 2.4 Prepare a sample by diluting 50 μ L of culture medium with 50–150 μ L of 1X dilution buffer (prepared in step 2.3) in a microfuge tube.

Note: To perform the detection assay on the maximum number of samples, use 50 μ L of the 1X dilution buffer, and follow rest of protocol as indicated (see **General Guidelines**).
- 2.5 Heat the diluted sample (prepared in step 2.4) at 65°C for 30 minutes, then cool on ice to room temperature.
- 2.6 Add 50 μ L of diluted sample (from step 2.5) to luminometer microplate wells.
- 2.7 Add 50 μ L of assay buffer (from step 2.2) per well, and incubate for 5 minutes at room temperature.
- 2.8 Add 50 μ L of working reaction buffer (from step 2.2) per well, and incubate for 20 minutes at room temperature.
- 2.9 Place the plate in a luminometer and measure chemiluminescence for 0.1–1 second per well.

Preparing Extract for Non-Secreted Placental Alkaline Phosphatase

This procedure is for adherent cells. You can lyse non-adherent cells by pelleting and covering them with sufficient 1X dilution buffer/0.2% Triton® X-100, followed by repeated pipetting.

- 3.1 Prepare 250 μ L of 1X lysis buffer per 60 mm plate by diluting 5X dilution buffer (Component C) 1:5 in deionized water. Add Triton® X-100 to a final concentration of 0.2% (v/v).
- 3.2 Rinse cells twice with PBS, add lysis buffer (prepared in step 3.1), and detach from plate with a cell scraper.
- 3.3 Prepare the extract by repeated pipetting, and transfer to a microcentrifuge tube. Centrifuge for 2 minutes to pellet cell debris.

- 3.4 Transfer the supernatant containing the extract to a fresh tube. Use the extract immediately or store at -80°C .
- 3.5 Aliquot 30 μL of cell extract (from step 3.4) into a microfuge tube and add 370 μL of 1X dilution buffer (for tube assays), or use 15 μL of extract with 185 μL of 1X dilution buffer (for microplate assays).
- 3.6 Proceed with the appropriate Detection Protocol, starting at step 1.5 or 2.5.

Direct Lysis Procedure for Microplate Cultures

This procedure is for adherent cells which express non-secreted placental alkaline phosphatase, cultured in 96-well tissue culture-treated luminometer plates. Heat inactivation is not effective with this protocol.

- 4.1 To prepare working reaction buffer (50 μL /well), dilute sufficient CSPD[®] substrate (Component D) 1:20 in reaction buffer (Component B).
- 4.2 Prepare 10 μL of 1X lysis buffer per well by diluting 5X dilution buffer (Component C) 1:5 in deionized water, and add Triton[®] X-100 to a final concentration of 0.2% (v/v). Prepare an additional 40 μL of 1X dilution buffer per well.
- 4.3 Rinse the wells of a 96-well tissue culture-treated luminometer plate once with PBS.
- 4.4 Add 10 μL of lysis buffer (from step 4.2) per well, and incubate for 10 minutes at room temperature.
- 4.5 Add 40 μL of 1X dilution buffer (from step 4.2) per well.
- 4.6 Add 50 μL of assay buffer (Component A) per well, and incubate for 5 minutes at room temperature.
- 4.7 Add 50 μL of working reaction buffer (prepared in step 4.1) per well, and incubate for 20 minutes at room temperature.
- 4.8 Place the plate in luminometer and measure chemiluminescence for 0.1–1 second per well.

References

1. Gene 66, 1 (1988); 2. Meth Enzymol 216, 362 (1992); 3. BioTechniques 17, 172 (1994); 4. Clin Chem 42, 1542 (1996); 5. J Endocrin 193, 421 (2007); 6. J Biol Chem 271, 7019 (1996); 7. Mol Cell Biol 15, 4272 (1995); 8. J Biol Chem 272, 791 (1997); 9. Int J Cancer 27, 637 (1981); 10. J Biol Chem 280, 25111 (2005); 11. Proc Natl Acad Sci USA 98, 3756 (2001); 12. J Neurosci 23, 4420 (2003); 13. Cell Research 15, 111 (2005); 14. J Virol 75, 3391 (2001); 15. J Biol Chem 281, 8205 (2006); 16. Antimicrob Agents Chemother 48, 2876 (2006); 17. J Gen Virol 87, 2297 (2006); 18. J Virol 77, 6823 (2003); 19. J Virol 76, 2075 (2002); 20. J Gen Virol 80, 1241 (1999); 21. J Gene Med 5, 773 (2003); 22. J Gene Med 6, 111 (2004); 23. J Virol 72, 6770 (1998); 24. Human Gene Therapy 13, 1611 (2002); 25. DNA Cell Biol 22, 807 (2003); 26. J Gen Virol 84, 781 (2003); 27. Mol Endocrinol 18, 150 (2004); 28. Mol Cell 2, 505 (1998); 29. J Exp Med 199, 1201 (2004); 30. Mol Biol Cell 19, 722 (2008); 31. J Cell Biol 179, 1123 (2007).

Product List

Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
N10559	NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection Kit *200 assays*	1 kit
N10560	NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection Kit *1200 assays*	1 kit

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