invitrogen™

NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection System 2.0

Catalog nos. N10577, N10578

Table 1. Contents and storage information.

Material	Composition	N10577	N10578	Storage	Stability
NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection Kit 2.0 2–6°C Components					
Assay buffer (Component A)	Contains a proprietary mixture of non-placental alkaline phosphatase inhibitors	14 mL	60 mL	• 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 CSPD® substrate and Emerald-III™ luminescence enhancer	14 mL	60 mL		
NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection Kit 2.0 ≤–20°C Components					
Control enzyme	0.3 ng/µL purified human placental alkaline phosphatase in 150 mM Tris (pH 7.8), 50 mM NaCl, 50% glycerol	50 µL	50 µL	• ≤–20˚C	When stored as directed, this kit is stable for 1 year.
Number of assays: Sufficient material is supplied for 192 (Cat. no. N10577) or 960 (Cat. no. N10578) microplate assays based on the protocol below.					

Introduction

The NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection System 2.0 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 medium and detected by testing aliquots of the medium, leaving the 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 PLAP (Non-Secreted) Detection Assay Performed Directly in the Cell Culture Microplate).

Description of the System

The next-generation NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection System 2.0 incorporates CSPD® chemiluminescent substrate and the next generation Emerald-III™ luminescence enhancer for high sensitivity and wide dynamic range. This new detection assay provides higher signal intensity and higher assay signal/noise performance than the original assay system. All the reagents in the new assay system are provided ready-to-use without the need for any reagent preparation steps, which allows fewer assay steps and shorter assay duration. The original NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection System[3,4] has been used for the detection of secreted placental alkaline phosphatase reporter enzyme in cell culture media[5,6] and for the quantitation of non-secreted placental alkaline phosphatase in cell and tissue extracts.[7,8]

The NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection 2.0 assay is simple and rapid. Depending on the expression system, secreted placental alkaline phosphatase can be measured in as little as 6 hours after cell transfection. Cell culture medium is incubated first with an assay buffer that contains differential inhibitors of non-placental alkaline phosphatase (serum and endogenous cellular alkaline phosphatases) and then with the reaction buffer containing CSPD® substrate and Emerald-III™ light emission enhancer until maximum light signal is reached in approximately 20 minutes. The light emission kinetics provide a persistent glow signal, with light emission half-life of approximately 2.5 hours, enabling measurement over a wide time interval. Light emission is measured in a luminometer without the need for automated injection capability.

Chemiluminescent reporter assays for secreted placental alkaline phosphatase may be performed 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 the medium of non-transfected cells to establish an 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 medium.[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 used widely for the following applications. The new version 2.0 system is compatible with all of these applications.

- Reporter gene assays measuring gene expression in established cell lines[10] 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]
- Assaying serum samples from transgenic, transfected, or viral vector-infected animals to measure SEAP levels in mouse,[21] rat,[22] marmoset,[23] monkey,[24] and pig sera,[25] and in chicken egg allantoic fluid.[26]
- Sensitive detection of mouse SEAP protein (mSEAP),[21] which has been developed for improved SEAP protein stability in transgenic mice.
- 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]
- 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 reporter expression vector
- 96-well luminometer microplates (solid white)
- Microplate luminometer (single-mode or multi-mode)
- *Optional*: 96-well tissue culture-treated luminometer microplates (white with clear bottom)

Caution

Components A and B 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 at room temperature, unless otherwise indicated.
- The NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection System 2.0 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.
- We recommend using a single-mode luminometer or a multi-mode detection instrument set for luminescence measurement to measure the light emission from 96- or 384-well microplates. If using a multi-mode detection instrument, make sure that wavelength filters are not in use. Taking luminescence measurements with wavelength filters in place is not necessary in the absence of excitation light and because it reduces signal intensity, we do not recommended it.
- The assay can also be performed in a tube luminometer or a 384-well microplate format, with reagent volumes scaled as appropriate.
- If sample dilution is desired, we suggest using sterile water or standard Tris-saline solutions available in the laboratory.

Controls

Recommended positive and negative controls are listed below.

Positive Control

The control enzyme supplied with the kit, purified human placental alkaline phosphatase (hPLAP), 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 control enzyme dilution curve is not intended (or accurate) for absolute quantitation of reporter enzyme concentrations, as the specific activity of the control hPLAP enzyme and the reporter enzyme may differ significantly. Additional positive controls can include the use of control SEAP constructs that provide constitutive expression of reporter enzyme as a positive control for cell transfection.

The control hPLAP enzyme is approximately 0.3 ng/µL (0.75 U/mL) in 150 mM Tris (pH 7.8), 50 mM NaCl, 50% glycerol. Generate an enzyme dilution curve by serially diluting the control enzyme in the desired cell culture medium. Detection curve should range from approximately 1 pg/mL to 10 ng/mL.

Negative Control

Determine the total assay background by assaying a volume of culture medium from mock-transfected cells that is equivalent to that from transfected cells. In experiments involving induction of reporter expression, assay uninduced cells to determine the total assay background. Experimental results are typically expressed as a ratio of signal from experimental cells to assay background (signal/background) or as results from induced cells normalized to results obtained with uninduced cells.

Experimental Protocols

SEAP Detection Assay Using a Separate Assay Microplate

This procedure is for detecting secreted placental alkaline phosphatase (SEAP) in 25 µL of conditioned cell culture medium removed from the cell culture microplate.

1.1 Equilibrate the Assay Buffer (Component A, 50 µL/well) and the Reaction Buffer (Component B, 50 µL/well) to room temperature.

1.2 Add 25 µL of cell culture medium (or control enzyme) to assay microplate wells.

1.3 Add 50 µL of Assay Buffer per well, and incubate the plate at 65°C for 5 minutes.

1.4 Add 50 µL of Reaction Buffer per well, and incubate the plate for 20 minutes.

1.5 Place microplate in a luminometer and measure for 0.1–1 second per well.

SEAP Detection Assay Performed Directly in the Cell Culture Microplate

This procedure is for detecting secreted placental alkaline phosphatase (SEAP) in 100 µL (or less) of conditioned cell culture medium directly in the cell culture microplate.

Note: The microplate must be white with a clear bottom or solid white.

2.1 Equilibrate the Assay Buffer (Component A, 50 µL/well) and the Reaction Buffer (Component B, 50 µL/well) to room temperature.

2.2 Add 50 µL of Assay Buffer directly to each well of a culture microplate containing 100 µL of cells and conditioned cell culture medium, and incubate at 65°C for 5 minutes.

2.3 Add 50 µL of Reaction Buffer per well, and incubate for 20 minutes.

2.4 Place the microplate in a luminometer and measure chemiluminescence for 0.1–1 second per well.

PLAP (Non-Secreted) Detection Assay Performed Directly in the Cell Culture Microplate

This procedure is for adherent cells expressing non-secreted placental alkaline phosphatase (PLAP), cultured in 96-well tissue culture-treated luminometer plates. If the culture medium is removed from the wells before performing the assay, heat inactivation is not necessary with this protocol. We recommend removing the culture medium before the assay, which increases the assay sensitivity ~20-fold, because the serum AP is responsible for the majority of the assay background.

3.1 Remove the culture medium from the assay wells.

3.2 Add 50 µL of Assay Buffer (Component A) per well, and incubate for 5 minutes at ambient temperature.

3.3 Add 50 µL of Reaction Buffer (Component B) per well, and incubate for 20 minutes.

3.4 Place the microplate in a 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
N10577	NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection Kit 2.0 *192 assays*	1 kit
N10578	NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection Kit 2.0 *960 assays*	1 kit

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