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Not for use in diagnostic procedures.

SEAP Reporter Gene Assay, chemiluminescent

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Chemiluminescent assay for the quantitative determination of secreted human placental alkaline phosphatase activity in culture supernatant of transfected cells

Cat. No. 11 779 842 001

Store the kit at +2 to +8°C.

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1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1a	red	SEAP Reporter Gene Assay, chemiluminescent, Alkaline Phosphate Substrate, CSPD	For chemiluminescent detection.	1 bottle, 1.3 ml
1b	black	SEAP Reporter Gene Assay, chemiluminescent, Substrate Buffer	Contains Emerald-II luminescence enhancer.	1 bottle, 25 ml
2	black	SEAP Reporter Gene Assay, chemiluminescent, Inactivation Buffer	▪ Contains a mixture of different alkaline phosphatase inhibitors. ▪ Ready-to-use solution.	1 bottle, 25 ml
3	blue	SEAP Reporter Gene Assay, chemiluminescent, Dilution Buffer	▪ For dilution purposes. ▪ Ready-to-use solution.	1 bottle, 100 ml
4	white	SEAP Reporter Gene Assay, chemiluminescent, Positive Control	Human placental alkaline phosphatase.	1 bottle, 4 Units

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1a	red	Alkaline Phosphate Substrate, CSPD	Store at +2 to +8°C.
1b	black	Substrate Buffer	
2	black	Inactivation Buffer	
3	blue	Dilution Buffer	
4	white	Positive Control	

1.3. Additional Equipment and Reagent required

For SEAP Reporter Gene Assay

- Scintillation counters, or
- Photographic films, or
- Automated or manual luminometers in tube or microplate format.
  Use luminometers with ultra-fast photon counters, such as the EG&G Berthold luminometers.
- Use only black or white microplates for the microplate format.
  Roche recommends using the black microplates.

For Preparation of Working Solutions

- Double-distilled water

1.4. Application

The SEAP Reporter Gene Assay is used to quantitatively measure secreted human placental alkaline phosphatase activity in culture supernatants of transfected cells.

1.5. Preparation Time

Assay Time

Approximately 60 minutes.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Culture medium from transfected eukaryotic cells.

General Considerations

Kinetics of Light Reaction

At low to intermediate concentrations of alkaline phosphatase (<100 pg), the light signal remains almost constant for at least one hour (Fig. 1). When exceeding 100 pg, due to substrate depletion, the signal intensity decreases with a half-life depending on the concentration of alkaline phosphatase. To combine maximum linear range and maximum sensitivity, it is optimal to measure 10 minutes after addition of Substrate Reagent.

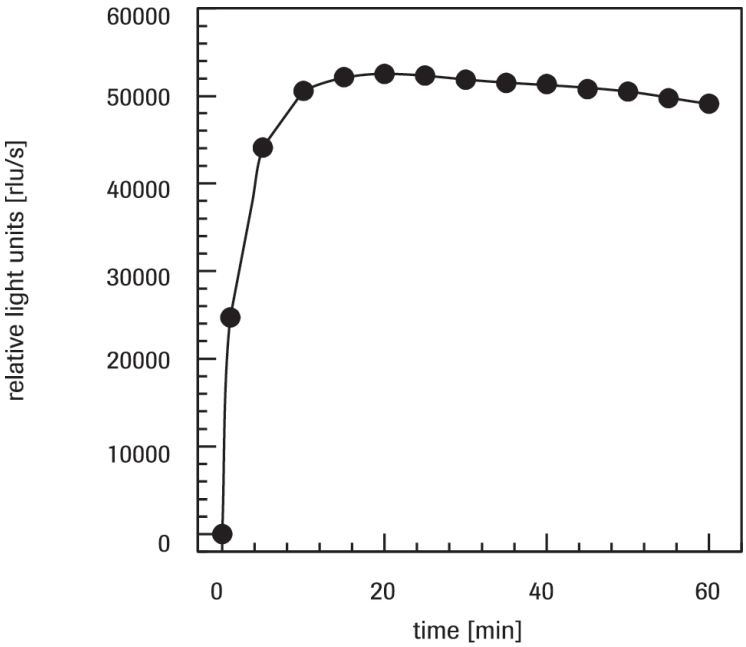

Fig. 1: Kinetics of light reaction. 100 pg intestinal alkaline phosphatase in a volume of 50 µl was detected in a black microplate according to the SEAP Assay, see section Protocols. The light signal was measured on a Berthold LB 96 P Luminometer with 1 second integration time.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Prepare the working solutions according to the following table.

⚠ ***To avoid confusion, label each solution with the appropriate solution number (solutions 1 to 4).***

Always use double-distilled water for reconstitution and dilution of reagents.

Solution	Content	Reconstitution/Preparation of Working Solution	Storage and Stability	For use in...
1	Substrate Reagent	▪ To prepare 1 ml, mix 50 µl Alkaline Phosphatase Substrate (Bottle 1a) and 950 µl Substrate Buffer (Bottle 1b). ▪ 50 µl substrate Reagent is needed per well.	Always prepare fresh before use.	Preparation of Substrate Reagent.
2	Inactivation Buffer	Ready-to-use solution.	Store at +2 to +8°C.	SEAP assay
3	Dilution Buffer	Ready-to-use solution.	Store at +2 to +8°C.	SEAP assay
4	Positive Control	▪ Reconstitute Positive Control (Bottle 4) with 1 ml double-distilled water. ▪ Solution 4 will contain 4 units/ml placental alkaline phosphatase (approximately 0.1 mg/ml).	Store 1 week at +2 to +8°C, or 3 months at -15 to -25°C in aliquots. ⚠ ***Avoid repeated freezing and thawing.***	Calibration curve

2.2. Protocols

SEAP Assay

Use the following standard volumes for the SEAP Reporter Gene Assay. Changing relative amounts and concentrations may result in reduced sensitivity. If the recommended volumes cannot be used, for example, due to fixed instrument settings, adjust all volumes to the same ratio.

1. Prepare the following sample volumes.

Assay Component	Volume/Test (MP Assay) [µl]	Volume/Test (Tube Assay) [µl]
Sample, diluted 1:4 with Dilution Buffer	50	100
Inactivation Buffer	50	100
Substrate Reagent	50	100

⚠ ***Fully Equilibrate Substrate Reagent and sample to +15 to +25°C before starting the test. Reagents with different lot numbers must not be used in one assay series.***

2. Centrifuge culture supernatant from transfected cells or from control cells to pellet any debris.
   - If necessary, store samples at -15 to -25°C.

3. In a microfuge tube, dilute sample 1:4 with Dilution Buffer (solution 3; e.g., 50 µl cell culture medium plus 150 µl Dilution Buffer).
   - Seal the tube and incubate in a water bath for 30 minutes at +65°C.

4. Centrifuge sample in a microfuge for 30 seconds at +15 to +25°C at full speed.
   - Transfer supernatant to an ice bath.

5. **Microplate assay**: Transfer 50 µl of heat-inactivated sample to a microplate (black or white) and add 50 µl Inactivation Buffer (solution 2).
   - After a 5 minute incubation period at +15 to +25°C, add 50 µl Substrate Reagent.
   - The addition of the Substrate Reagent (solution 1) should be timed using the same interval as the luminometer/LSC reads the samples.

6. **Tube assay**: Transfer 100 µl of heat-inactivated sample to a tube and add 100 µl Inactivation Buffer (solution 2).
   - After a 5 minute incubation period at +15 to +25°C, add 100 µl Substrate Reagent.
   - The addition of the Substrate Reagent (solution 1) should be timed using the same interval as the luminometer/LSC reads the samples.

7. Incubate for 10 minutes at +15 to +25°C with gentle rocking.
   - Transfer microplates/tubes to the luminometer or liquid scintillation counter.

8. Integrate light signal for 1 to 5 seconds.
   - The light emission peaks within 10 minutes and then remains practically constant for at least 60 minutes (Fig. 1, **Kinetics of Light Reaction**).

Calibration Curve

To set up a calibration curve, dilute the human placental alkaline phosphatase Positive Control (solution 4) with Dilution Buffer (solution 3). For better resolution, position the highest standard concentration in the signal range generated by cells transfected with the control vector. The full range of the SEAP assay is shown in Figure 2, see **Results Field**.
For internal standardization, add a small amount of the Positive Control (in the range of the expected signal) to one aliquot of the undiluted sample. All following steps must be performed in the same way. After subtraction of the background signal, the SEAP content of the culture medium can be estimated from the difference in signal of the sample with and without exogenic SEAP.

2.3. Parameters

Detection range

10 fg to 1 ng alkaline phosphatase.
The exact detection limit depends on the measuring device and conditions used.

Sensitivity

Approximately 20 fg alkaline phosphatase.

Specificity

The SEAP Reporter Gene Assay is designed to specifically measure secreted placental alkaline phosphatase in conditioned cell culture medium from transfected cells. This is achieved by taking advantage of the special features of SEAP. Contaminating alkaline phosphatase activity, which is generally present in culture medium, is almost completely eliminated by a heat inactivation step and specific inhibitors, without significantly affecting SEAP activity.

3. Results

Interpretation of Results

After completing the protocol, plot the chemiluminescence signals obtained on the Y-axis against the SEAP standard concentrations on the X-axis to obtain a calibration curve (Fig. 2).

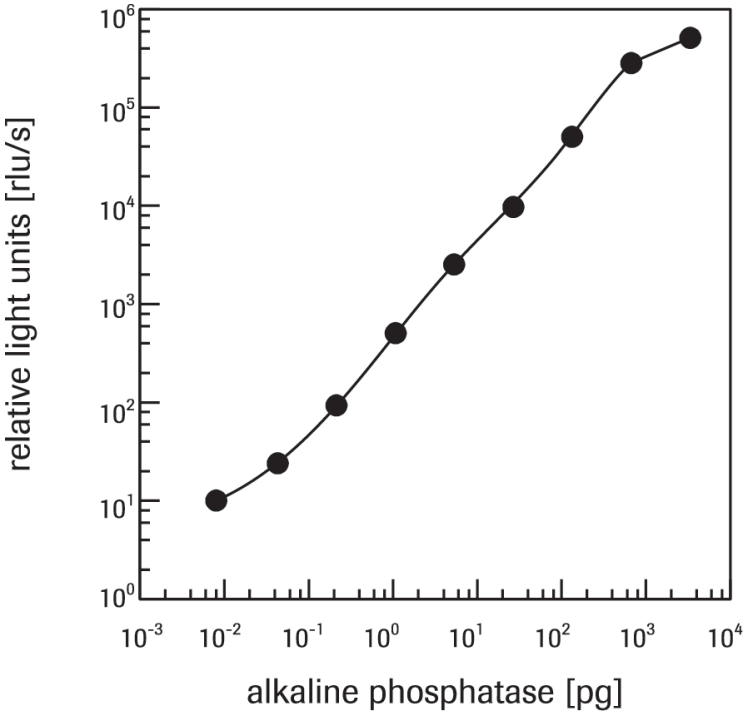

Fig. 2: Alkaline phosphatase calibration curve. Serial dilutions of human placental alkaline phosphatase in a volume of 50 µl were applied to a black microplate and incubated with 50 µl of Inactivation Buffer for 5 minutes. Light reaction was initiated by the addition of 50 µl Substrate Reagent. 10 minutes after addition of Substrate Reagent, the light signal was measured on a Berthold LB 96 P Luminometer with 1 second integration time.

SEAP concentrations of unknown samples are obtained by plotting the observed signal on the Y-axis, extrapolating to meet the calibration curve, and reading the resulting SEAP concentration from the X-axis. To obtain reliable results, the signal of the sample should be within the linear portion of the calibration curve.

To allow direct, quantitative comparison of data obtained in independent experiments, a separate calibration curve must be established for each assay series.

4. Additional Information on this Product

4.1. Test Principle

Quantification of secreted alkaline phosphatase (SEAP) has become a powerful tool for investigating promotor activity in transfected eukaryotic cells. The SEAP gene product is secreted from transfected cells and is thus easily detected in a sample of culture medium, without destroying cells and without time-consuming sample preparation. The SEAP Reporter Gene Assay, chemiluminescent, based on CSPD, provides a convenient and highly sensitive method for the quantification of transcriptional activity.

Test Principle

CSPD 3-(4-methoxyspiro{1,2-dioxetane-3,2′(5′-chloro)-tricyclo[3.3.1.(3,7)]decan}-4-yl)phenyl phosphate] is dephosphorylated by alkaline phosphatase (Fig. 3). The resulting unstable dioxetane anion decomposes and emits light with its maximum activity at a wavelength of 477 nm. Adamantyl-1,2-dioxetanes are well established in the detection of alkaline phosphatase and are commonly used as labels in immunoassays (ELISA) and protein and nucleic acid blotting techniques. Chemiluminescence-enhancing reagents improve the quantum yield of the excited state more than 500-fold. The light signal, quantitated in a tube or microplate luminometer or in a scintillation counter (single photon mode), is linear up to 5 orders of magnitude and proportional to the concentration of alkaline phosphatase.

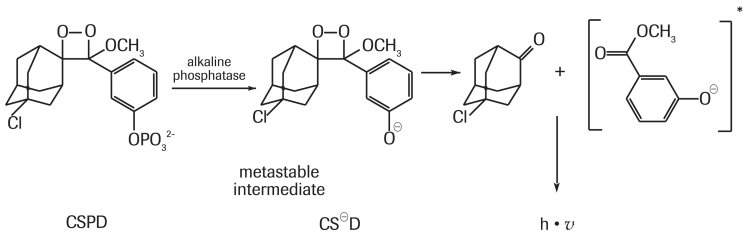

Fig. 3: Test principle

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

5.3. Trademarks

All product names and trademarks are the property of their respective owners.

5.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

5.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany