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Print

BM Chemiluminescence ELISA Substrate (AP)

Chemiluminescent substrate system for highly sensitive detection of alkaline phosphatase in ELISA and SEAP reporter gene assays

Cat. No. 11 759 779 001

1000 ELISA tests (150 ml substrate reagent)
600 SEAP tests (60 ml substrate reagent)

Version 05
Content version: April 2016

Store at +2 to +8°C

1. Product overview

Contents

The kit contains reagents for performing 1000 ELISA tests (150 µl/assay) or 600 SEAP reporter gene assays (MP-formate).

Bottle	Label	Contents
1 red	Alkaline Phosphatase Substrate, CSPD	1.5 ml
2 black	Enhancer	15 ml
3 green	Assay buffer	2× 75 ml

Introduction

Adamantyl-1,2-dioxetanes are well established in the detection of alkaline phosphatase labels in immunoassays (ELISA) as well as in protein and nucleic acid blotting. More recently, quantification of secreted alkaline phosphatase (SEAP) has become a powerful tool for investigating promotor activity in transfected eukaryotic cells (1, 2). The SEAP gene product is secreted from transfected cells and is thus easily detected in a sample of culture medium without destroying cells and time-consuming sample preparation.

The BM Chemiluminescence ELISA Substrate (AP) based on CSPD provides a convenient and highly sensitive method for the detection of alkaline phosphatase in ELISA and SEAP reporter gene assays.

Test principle

CSPD (3-(4-methoxyspiro [1,2-dioxetane-3,2´(5´-chloro)-tricyclo[3.3.1.1^{3,7}]decan]-4-yl)phenylphosphate) is dephosphorylated by alkaline phosphatase (AP). The resulting instable dioxetane anion decomposes and emits light at a maximum wavelength of 477 nm. Chemiluminescence-enhancing reagents improve the quantum yield of the excited state by more than 500fold. 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.

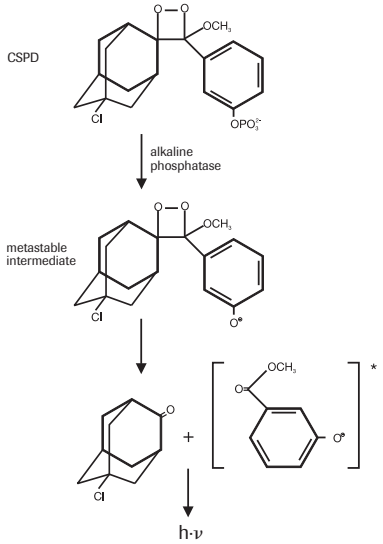

Fig. 1: Test principle

Specificity

The AP assay system is designed for measuring alkaline phosphatase of various sources (*e.g.,* intestine, placenta, milk, bacteria).

Detection range

The detection range for alkaline phosphatase is between 10 fg and 1 ng (fig. 2). However, the exact detection limit depends on the measuring device and the measuring conditions used.

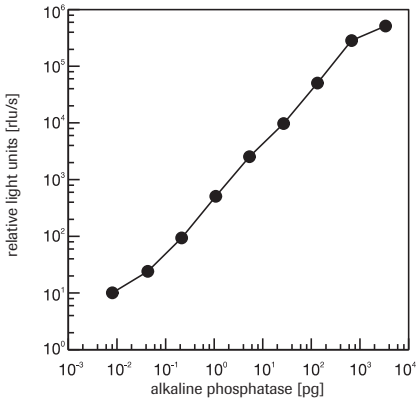

Fig. 2: Alkaline phosphatase (intestinal) calibration curve. Serial dilutions of alkaline phosphatase in a volume of 10 µl were applied to a black microtiter plate. The detection was performed with 150 µl substrate reagent according to section 2.1. 10 min after addition of substrate reagent the light signal was measured on a Berthold LB 96 P Luminometer with 1 s integration time.

Kinetics of light reaction

At intermediate and low concentrations of alkaline phosphatase (< 100 pg) the light signal is almost constant for at least one hour (fig. 3). When exceeding 100 pg, due to substrate depletion, the signal intensity decreases with a half-life depending on the concentrations of alkaline phosphatase. To combine maximum linear range and maximum sensitivity it is optimal to measure 10 min after addition of substrate reagent.

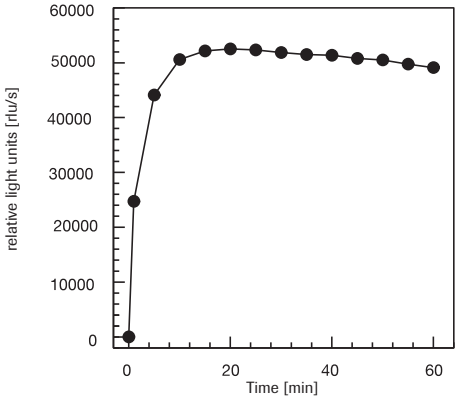

Fig. 3: Kinetics of light reaction. 100 pg alkaline phosphatase (intestinal) in a volume of 10 µl was detected in a black microtiter plate with 150 µl substrate reagent according to section 2.1. The light signal was measured on a Berthold LB 96 P Luminometer with 1 s integration time.

Storage/ stability

The kit is stable at +2 to +8°C until the expiration date printed on the label.

Preparation of working solution

The substrate reagent is composed of AP-substrate (bottle 1), Enhancer (bottle 2) and Assay buffer (bottle 3). Prepare substrate reagent freshly before use.

The ratio of the components depends on the application.

The substrate reagent is stable at least for one day at +2 to +8°C. To prepare 1 ml of substrate reagent the following amounts are needed:

	Standard ELISA	Reporter Gene Assay
AP-substrate (CSPD)	10 µl	25 µl
Enhancer	100 µl	225 µl
Assay buffer	890 µl	750 µl
L-Homoarginine	-	15 mM
Volume needed per test*	150 µl	100 µl
Tests/kit	1000 tests	600 tests

* the volume might be adapted according to special formats

Advantage

Please refer to the following table.

Benefits	Feature
Sensitive	approx. 10 fg intestinal alkaline phosphatase
High dynamic measuring range	linear range of more than five orders of magnitude
Constant light emission	the assay produces a long-lasting light emission instead of a short peak kinetics
Safe	no radioactive isotopes are used

2. Procedures and required material

2.1 ELISA Protocol

Handling instructions

- The AP assay can be performed in all automated or manual luminometers in tube and microplate format as well as in liquid scintillation counters (LSC).
- When using the microplate format, black or white microplates must be used.
- The kit contains all reagents required for ELISA application.
- For SEAP reporter gene analysis dilution buffer (TBS) and L-homoarginine are additionally required

Procedure

Important: All reagents should be fully equilibrated to room temperature (+20 to +23°C) before starting the test. Reagents with different lot numbers must not be used in one test. Changing relative amounts and concentrations may result in reduced sensitivity.

- Follow your standard ELISA protocol as optimized for colorimetric detection. Apply sample, primary and secondary antibody in a volume of 150 ml each. Otherwise adapt the volume of assay reagent to the altered conditions.
- After incubation of the AP-labeled antibody discard the solution and wash three times (*e.g.*, with TBS), leaving the buffer in the wells for 0.5–5 min between each individual wash.
- Discard the wash solution and tap the MP on a lint-free, dry, absorbant cloth.

Manual application of the assay reagent

- Add 150 ml substrate reagent per well.
  Note: For best accuracy the addition of the substrate reagent should be timed in the same interval as the luminometer/LSC reads the samples.
- Incubate for 10 min at room temperature, while gently rocking.

Transfer MP to the luminometer. Integrate light production for 1–5 s.
Note: The light emission peaks after 10 min and keeps constant for at least 60 min (fig. 3).

Automatic application of the assay reagent

- Transfer MP to the luminometer.
- Inject 150 µl substrate reagent automatically in the same interval as the luminometer reads the samples.
- Incubate for 10 min at room temperature.
- Start integration of light reaction for 1–5 s.

2.2 SEAP Reporter gene assay

Additional reagents required

Dilution buffer: 25 mM Tris/HCl, 150 mM NaCl, pH 7.4 (TBS)

Procedure

Important: All reagents should be fully equilibrated to room temperature (+20 to +23°C) before starting the test. Reagents with different lot numbers must not be used in one test. Changing relative amounts and concentrations may result in reduced sensitivity.

Step	Action
1	Collect culture medium of transfected cells or control cells. ***Note***: If necessary store samples frozen at −15 to −25°C.
2	Dilute sample in a microfuge tube 1:4 with Dilution buffer (*e.g.,* 50 ml cell culture medium + 150 ml Dilution buffer), seal the cup and incubate in a water bath at +65°C for 30 min.
3	Collect sample by centrifugation in a microcentrifuge for 1 min at full speed and transfer sample to an ice bath (0 to +4°C).
4	Add a final concentration of 15 mM L-homoarginine (MW 224.7, 34 mg/10 ml) to the assay buffer. ***Note***: This inhibitor blocks contaminating isoforms of alkaline phosphatase (1).
5	Pipette 50 µl of heat-inactivated sample into a microplate (black or white) or a tube and start reaction with 100 µl substrate reagent. ***Note***: The addition of the substrate reagent should be timed in the same interval as the luminometer/LSC reads the samples.
6	Incubate for 10 min at room temperature, while gently rocking. Transfer MP/tubes to the luminometer or liquid scintillation counter.
7	Integrate light production for 1–5 s. ***Note***: The light emission peaks within 10 min and then keeps constant for at least 60 min (fig. 2).

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3. Appendix

3.1 References

1 Cullen B.R. & Malim M.H. (1992) *Methods Enzymol.* **216**, 362.
2 Bronstein I. et al. (1994) *Anal. Biochem.* **219**, 169.

3.2 Change to previous version

Editorial changes

3.3 Ordering Information

Product	Pack size	Cat. No.
BM Chemiluminescence ELISA Substrate (POD)	250 ml	11 582 950 001
Transfection Reagent (DOTAP)	2 ml (5× 0.4 ml) (5× 0.4 mg)	11 202 375 001
β-Gal Reporter Gene Assay, chemiluminescence	1 kit (500 tests)	11 758 241 001
Luciferase Reporter Gene Assay, high sensitivity	200 assays 1000 assays	11 669 893 001 11 814 036 001
β-Gal ELISA	1 kit (192 tests)	11 539 426 001
CAT ELISA	1 kit (192 tests)	11 363 727 001
hGH ELISA	1 kit (192 tests)	11 585 878 001
Geneticin (G418)	250 mg 1 g 5 g	11 464 973 001 11 464 981 001 11 464 990 001
Hygromycin B	1 g (20 ml)	10 843 555 001
Alkaline Phosphatase, EIA grade	3 mg 15 mg	10 567 744 001 10 567 752 001

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4. Short protocol

Working solutions

	ELISA	SEAP Reporter Gene Assay
Substrate reagent (ratios of 1 ml)	10 µl AP substrate (bottle 1) 100 µl enhancer) (bottle 2) 890 µl assay buffer (bottle 3)	25 µl AP substrate (bottle 1) 225 µl enhancer (bottle 2) 750 µl assay buffer (bottle 3) 3.4 mg/ml L-homoarginine

ELISA

Step	Procedure	Reagent/ Volume	Time/ Settings	Temp.
1	Follow your standard ELISA protocol as optimized for colorimetric detection until incubation with the AP-labeled antibody.			
2	Wash	TBS	3 times	RT
3	Enzyme reaction	150 µl substrate reagent	10 min	RT
4	Signal integration	–	1–5 s	–

SEAP Reporter Gene Assay

Step	Procedure	Reagent/ Volume	Time/ Settings	Temp.
1	Pre-equilibrate all reagents and samples fully to room temperature			RT
2	Collect sample	Conditioned culture medium	–	RT
3	Sample dilution	25 µl culture medium plus 75 µl TBS	1 : 4 dilution	RT
4	Heat inactivation	Diluted culture medium	30 min	65°C
5	Centrifugation	Heat-inactivated sample	1min, maximum speed	RT
6	Enzyme reaction	50 µl sample + 100 µl substrate reagent	10 min	RT
7	Signal integration	–	1–5 s	–

Contact and Support

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