SEAP Reporter Assay Kit

A staining kit to determine secreted embryonic alkaline phosphatase activity

Catalog code: rep-sap

http://www.invivogen.com/seap-reporter-assay

For research use only

Version 18K21-MM

PRODUCT INFORMATION

Contents

Component	Amount	Storage
10X Dilution Buffer	2 x 1 ml	4 °C
5X Assay Buffer	4 x 1 ml	4 °C
100 mM L-Homoarginine	4 x 1 ml	-20 °C
SEAP substrate	20 tablets	-20 °C

Shipping and storage

Kit is shipped at room temperature. Upon receipt, store each component at the appropriate temperature listed above.

Additional required materials to be supplied by user

- Centrifuge
- 37 °C incubator
- 65 °C incubator
- ELISA reader/spectrophotometer

INTRODUCTION

SEAP is a secreted form of embryonic alkaline phosphatase and thus can be easily detected in a sample of culture medium without destroying the cells and time-consuming sample preparation. SEAP catalyzes the hydrolysis of pNitrophenyl phosphate producing a yellow end product that can be read spectrophotometrically at 405-415 nm.

SEAP Reporter Assay Kit provides a convenient and highly sensitive method to determine the amount of SEAP produced by cells transfected with a SEAP-expression plasmid. Read through the entire procedure before starting to become familiar with the protocol as well as the volume of reagents that will be required for each experiment.

METHOD

Determine your needs

Volumes of each solution; dilution buffer, assay buffer and staining solution must be determined based on the volume of the sample. Refer to the table below.

Sample Volume	10 µl	20 µl	50 µl	100 µl
1x Dilution Buffer	50 µl	100 µl	250 µl	500 µl
1x Assay Buffer	100 µl	200 µl	500 µl	1 ml
100 mM L-Homoraginine	20 µl	40 µl	100 µl	200 µl
Staining Solution	20 µl	40 µl	100 µl	200 µl
H_2O	20 µl	40 µl	100 µl	200 µl
Total Volume	220 µl	440 µl	1.1 ml	2.2 ml

Preparation of solutions

- 1X Dilution Buffer

Volume	1 ml	2.5 ml	5 ml	7.5 ml
10x Dilution Buffer	100 µl	250 µl	500 µl	750 µl
H_2O	900 µl	2.25 ml	4.5 ml	6.75 ml

- 1X Assay Buffer

Volume	1 ml	5 ml	10 ml	15 ml
5X Assay Buffer	200 µl	1 ml	2 ml	3 ml
H_2O	800 µl	4 ml	8 ml	12 ml

- Staining Solution

Volume	300 µl	1 ml	2 ml	3 ml
SEAP substrate	2 tablets	7 tablets	13 tablets	19 tablets
H_2O	320 µl	1.1 ml	2.05 ml	3 ml

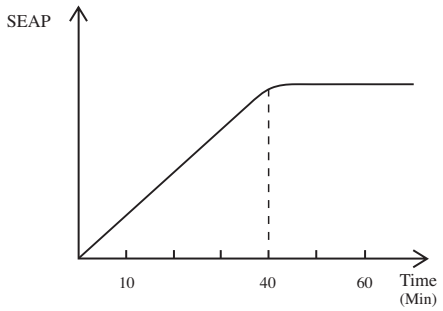
SEAP quantification

The following protocol refers to the use of 96-well plates. Vary your procedure accordingly depending on volumes of samples and reagents needed. (See «Determine your needs»)

1. Collect 1 ml of culture medium of transfected cells or control cells.
2. Centrifuge 5 min at 10,000 rpm.
3. Transfer 500 µl of supernatant to a microfuge tube to eliminate cell debris.

Note: Supernatants can be stored at -20 °C for further use.

4. Transfer 10 µl of supernatant in a well of a 96-well plate.
5. Add 10 µl of culture medium in a control well as a blank.
6. Heat sample to 65 °C for 5-10 min to inhibit endogenous alkaline phosphatase.
7. Add to each well 50 µl of 1X dilution buffer, 100 µl of 1X assay buffer, 20 µl of 100 mM L-homoarginine and 20 µl H_2O.
8. Incubate at 37 °C for 10 min.
9. Add 20 µl of staining solution.
10. Incubate at 37 °C for 10 min in the dark and take an initial reading of the $OD_{405-415}$. Then re-incubate at 37 °C in the dark and take additional readings as needed over a maximum incubation period of 60 min.



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