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Product Information

Alkaline Phosphatase Detection Kit, Fluorescence

Catalog Number **APF**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The gene for alkaline phosphatase (AP) commonly serves as a reporter gene and is used to determine the strength of promoters and enhancers, define the role of transcription factors, assess transfection efficiency, and measure the success of molecular cloning attempts.

The Alkaline Phosphatase Detection Kit offers the following advantages:

- The assay is safe, easy-to-use, and provides rapid and reproducible results.
- The enzyme is highly stable.
- A mutated version for the human placental alkaline phosphatase (SEAP) was designed to be secreted out of the cells. Its activity in the same cell sample can be measured nondestructively and repeatedly over time using an aliquot of the culture medium. This saves the time required for cell extract preparations.

The assay used is fluorometric and is at least 10–100 times more sensitive than the colorimetric assay. It is linear over a wide range of enzyme concentrations, making it particularly well suited for comparative analysis.

Components

The kit provides sufficient reagents to perform 300 reactions using a 200 µl reaction volume.

Fluorescent Assay Buffer (Catalog Number B6558)	50 ml
Dilution Buffer (Catalog Number B6433)	6 ml
50 µl of alkaline phosphatase control enzyme (0.1 mg/ml, Catalog Number C9361)	1 vial
4-Methylumbelliferyl phosphate disodium (Catalog Number M8168)	2 × 1 mg

Equipment and reagents required but not provided

- Fluorometer
- Fluorometer cuvettes or fluorometer 96 well plate (Catalog Numbers P8741, P8616, or equivalent)
- Water, Molecular Biology Reagent (Catalog Number W4502)
- Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham, without phenol red (Catalog Number D6434). The use of this medium is recommended for cells transfected with secreted alkaline phosphatase.
- L-Homoarginine hydrochloride (Catalog Number H1007, Optional)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the kit at –20 °C.

Procedure

These instructions are designed for a 96 well plate format. If the assay is performed in a larger format, the volumes should be increased accordingly.

Notes: It is recommended to add a negative and a positive control for each experiment:

- A negative control will indicate the background level. A medium, which was incubated at 65 °C, with no secreted AP can serve as a negative control for the detection of secreted alkaline phosphatase. A cell lysate of non-transfected cells, or cells transfected with non-relevant plasmid, can serve as a negative control for the detection of non-secreted alkaline phosphatase.
- A positive control will confirm the assay conditions are accurate. Moreover, a serial dilution of the provided control enzyme can indicate the linear range of the activity detection. Starting with a 1:100 dilution is recommended. The activity of the control enzyme is 20–50 units/mg of protein, where one unit will hydrolyze 1 mmole of *p*-nitrophenyl phosphate per minute at pH 10.4 at 37 °C.

1. Prepare Substrate Solution - Dissolve 1 mg of 4-methylumbelliferyl phosphate disodium salt in 330 µl of deionized water (10 mM). Mix well. It is recommended to aliquot the solution into amber containers and store at –20 °C when not in use.

2. Thaw the buffers - Allow the amount of buffer, sufficient for the entire experiment, to equilibrate to room temperature. After thawing the Fluorescent Assay Buffer and the Dilution Buffer for the first time, the buffers can be stored at 2–8 °C.

3. Prepare samples for assay - For secreted alkaline phosphatase, collect medium. For non-secreted alkaline phosphatase, collect cell lysate. It is highly recommended to use CelLytic™ products.

4. Incubate samples at 65 °C for 15–30 minutes. If using a 96 well plate, place 20 µl of each sample into each well. Add a sample of negative control. The incubation at 65 °C is performed to minimize background activity and phosphatase activity. The majority of the protein products of alkaline phosphatase genes, cloned as reporter genes, are stable at 65 °C. If you are not sure of the stability of your reporter protein, optimize this step by incubating your sample at 65 °C, and sampling the reporter protein every few minutes up to 30 minutes. Alternatively, you may decrease the heat inactivation temperature.

5. Cool the samples to room temperature. This step can be performed by a 2 minute incubation on ice followed by sample equilibration to room temperature.

6. Add buffers - To each well, add 20 µl of Dilution Buffer and 160 µl of Fluorescent Assay Buffer. Alternatively, prepare a bulk mixture containing Dilution Buffer and Fluorescent Assay Buffer at 1:8 ratio and add 180 µl to each well.

7. Add substrate - To each well, add 1 µl of the 10 mM substrate solution and mix. Alternatively, dilute the substrate solution 4× (1 volume of substrate and 3 volumes of deionized water). Add 4 µl of the diluted substrate to each well and mix.

8. Read the plate - The fluorometer should be set to 360 nm excitation and 440 nm emission. Since the activity of the enzyme increases with time, it is recommended to read the plate at several time points after the addition of the substrate. Between readings, incubate the plate in the dark at room temperature.

Troubleshooting

If the detected AP activity is too high:

- Dilute the sample or assay a smaller volume and perform the same changes with the negative control.
- If the detected activity in the negative control is too high, extend the 65 °C incubation step. Another option, for cases in which you detect an alkaline phosphatase resistant to L-homoarginine (e.g., SEAP), is to add the reagent L-homoarginine hydrochloride to a final concentration of 10–15 mM.
- If the background AP activity is high in media samples, minimize the percentage of serum in the media (as low as possible), since the presence of serum in the culture media increases background activity.

If the AP activity detected is low:

- Ensure that the transfection conditions were optimal, including the nature of the DNA vector and its purity, number of cells in the plate, etc.
- For detection of secreted AP, use a medium without phenol red.
- Increase the time period from cell transfection to cell harvest/medium collection to allow longer expression of the gene.
- Verify that there is no inhibitory factor in the detected sample by comparing the activity of positive control in the presence or absence of the medium/lysate to be detected.

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