Tribioscience

Alkaline Phosphatase (ALP) Fluorescent Substrate System (Catalog# TBS5025)

A single Stabilized Solution Ready-to-use for ALP-based biochemical and Immunoassays

Description

4-Methylumbelliferyl Phosphate (4-MUP) is a substrate for an alkaline phosphatase that forms the soluble highly fluorescent reaction product methylumbelliferone. The 4-MUP Liquid Substrate System combines 4-Methylumbelliferyl Phosphate (4MUP) and buffer in a single solution for detecting alkaline phosphatase enzyme activity in the culture media or ELISA. Fluorescence can be measured with excitation at 360 nm and emission at 450 nm. Also, the fluorescent product may also be observed using a UV light source. This substrate is about 10 times more sensitive than the alkaline phosphatase substrate BCIP/NBT.

Features

Flexible: Can be used for 96-well and 384-well plate.
Sensitivity: More sensitive than another alkaline phosphatase substrate like BCIP/NBT.
Time saving: Just add and read manner.

Application

- ALP substrate – for detection of alkaline phosphatase activity on liquid system.
- Used for immunoassays like ELISA and western blot.
- Custom packaging and bulk purchase information is available upon request.

Kit Contents

Part	Unit Size
4-MUP Substrate Solution	20 mL
ALP Standard (50U/L)	100 µL

Storage:
Store at -20°C and protect from light for up to 2 years.

Recommended Procedures

ALP activity

Warm up 4-MUP substrate solution to room temperature before testing.
1. Prepare samples and ALP standards according to your established procedures.
2. To each sample and AP standard, add 50 µL of 4-MUP Substrate Solution. (Note: This quantity should be optimized with pilot test.).
3. Record the fluorometric values (Ex360nm/Em440nm) for each sample and ALP standard at the desired intervals (kinetics assay) or upon termination of the reaction (endpoint assay).
4. Prepare ALP standard curve by plot of the concentrations (or amounts) of the ALP standards v. fluorometric values.
5. Using the fluorometric value for each sample, determine the ALP concentration by extrapolation on the standard curve generated in Step 4.

Western blotting

1. Remove nitrocellulose membrane from the transfer apparatus and block nonspecific sites with Blocking Buffer for 10-30minutes at room temperature with shaking.
2. Add the primary antibody and incubate membrane for 1 hour while shaking.
3. Wash the membrane with TBST.
4. Add the ALP-conjugated secondary antibody and incubate membrane for 1 hour at room temperature with shaking.
5. Wash membrane with TBST.
6. Add 4-MUP Substrate Solution to cover the membrane and incubate 5-15 min.
7. Visualize membrane by fluorescence.

Relative Products

Cell Lysis Buffer(10x) (TBS5001)
Protein Assay kit (TBS2005)
Ultra-sensitive TMB substrate (TBS5021)
HRP Fluorescent System (TBS5026)
Stop Solution for TMB Substrate (TBS5030)
Tryptase Activity Assay (TBS2101)
β-Hexosaminidase Activity Assay (TBS2105)
Cytochrome C Oxidase Activity Assay (TBS2115)
Cell Nuclear Extract kit (TBS6025)

For research use only.