CREATIVE BIOMART®
Assay Kit

Malachite Green Phosphate Assay Kit

Product Information

Common Name

Phosphate

Cat.No. Kit-0555

Product Overview

Lactate Dehydrogenase Assay Kits provide both fluorescence and absorbance-based method for detecting either L-LDH or D-LDH in biological samples such as serum, plasma, urine, as well as in cell culture samples. In the enzyme coupled assay, LDH is proportionally related to the concentration of NADH that is specifically monitored by a fluorogenic NADH sensor. This assay kit is specific for L-LDH. The signal can easily read by an absorbance microplate reader at ~575 nm or at the absorbance ratio of A575nm/A605nm to increase assay sensitivity. With the L-Lactate Dehydrogenase Assay Kit, we were able to detect as little as 3mU/mL LLDH in a 100 µL reaction volume.

Description

The Malachite Green Phosphate Assay Kit is based on quantification of the green complex formed between Malachite Green, molybdate and free orthophosphate. The rapid color formation from the reaction can be conveniently measured on a spectrophotometer (600 - 660 nm) or on a plate reader. The non-radioactive colorimetric assay kits have been optimized to offer superior sensitivity and prolonged shelf life. The assay is simple and fast, involving a single addition step for phosphate determination. Assays can be executed in tubes, cuvettes or multi-well plates. The assays can be conveniently performed in 96- and 384-well plates for high-throughput screening of enzyme inhibitors.

Applications

Phosphatase Assays: liberation of phosphate from peptide, protein or small molecule substrate.
Lipase Assays: liberation of phosphate from phospholipids
Nucleoside Triphosphatase Assays: liberation of phosphate from nucleoside triphosphates (ATP, GTP, TTP, CTP etc).
Quantitation of Phosphate in phospholipids, proteins and DNAs, etc.
Drug Discovery: high-throughput screen for phosphatase inhibitors.

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.org
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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Storage

The kit is shipped at ambient temperature. The reagents and standard are stable for one year when stored at 4°C.

Kit Components

Reagent A: 50 mL
Reagent B: 1 mL
Standard: 1mL 1 mM phosphate

Technical Notes

a. Incubation time. The chromogenic reaction is completed within 30 min at room temperature. Read OD values at 30 min.
b. Precipitation may occur at high concentrations of phosphate (>100 μM), or in the presence of high concentrations of e.g. proteins and metals.
If precipitation occurs, perform a series dilution of sample in H2O, run the assay and determine the dilution factor from wells with no precipitation. Repeat assays using diluted samples.
c. Enzyme reaction buffer. Because any exogenous free phosphate would interfere with the assay, it is important to ensure that the protein preparation, the reaction buffer and lab wares employed in the assay should not contain free phosphate. This can be conveniently checked by adding the Working Reagent to the buffer and measuring the color formation.
d. Liquid disposal. The assay mixture contains 0.4 M sulfuric acid. It is recommended that the waste liquid be neutralized with equal volume of 1 N NaOH prior to disposal.

Preparation

Reagent Preparation. Each assay requires 20 μL Working Reagent. Prepare enough Working Reagent by mixing 100 vol of Reagent A and 1 vol of Reagent B (e.g. 5 mL Reagent A and 50 μL Reagent B). Working Reagent is stable for at least 1 day at room temperature.
Important: The reagent must be brought to room temperature before use. Before each assay, it is important to check that all enzyme preparations and assay buffers do not contain free phosphate. This can be conveniently done by adding 20 μL of the Working Reagent to 80 μL sample solution. The blank OD values at 620 nm should be lower than 0.2. If the OD readings are higher than 0.2,

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check water phosphate level. Double distilled water usually have OD readings lower than 0.1. Lab detergents may contain high levels of phosphate. Make sure that lab wares are free from contaminating phosphate after thorough washes.

Assay Protocol

1. Preparation of phosphate standards. Prepare a Premix solution containing 40 µM phosphate by pipetting 40 µL 1 mM phosphate standard to 960 µL distilled water or enzyme reaction buffer. Number the tubes.
Dilute standards as shown in the following Table. Pipette 80 µL standard in duplicate into wells of a clear-bottom 96-well plate. Add blank controls containing water or reaction buffer only.
No Premix + H2O Final Vol (µL) Phosphate Conc (µM) pmoles Phosphate in 50 µL
1 200µL + 0µL 200 40 2,000
2 160µL + 40µL 200 32 1,600
3 120µL + 80µL 200 24 1,200
4 80µL + 120µL 200 16 800
5 60µL + 140µL 200 12 600
6 40µL + 160µL 200 8 400
7 20µL + 180µL 200 4 200
8 0µL + 200µL 200 0 0

2. Transfer 80 µL test samples into separate wells of the plate.
Note: in the case of enzyme reactions, the reaction may be terminated by adding a specific inhibitor, or can be stopped directly by the addition of the Working Reagent. Dilution of reaction mixture may be necessary prior to the assay (see General Considerations). For ATPase or GTPase assays, the ATP or GTP concentration should be lower than 0.25 mM. If the reaction mixture contains > 0.25mM ATP or GTP, dilute samples in distilled water. For example, if the ATPase reaction contained 1 mM ATP, at the end of reaction dilute reaction mixture 4-fold in water prior to the assay.
3. Add 20 µL of Working Reagent to each well. Mix gently by tapping the plate.
4. Incubate for 30 min at room temperature for color development.
5. Measure absorbance at 600 nm - 660nm (620 nm) on a plate reader.
For assays in 384-well plates, the procedures are the same, except that the volume of the standard

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and sample solution should be 40 µL and that of the Working Reagent should be 10 µL.

Analysis

Plot OD620nm versus phosphate standard concentrations. Determine sample phosphate concentrations from the standard curve.

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