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ProductInformation

NDPase (ADPase, GDPase, UDPase) Assay Kit

Product Code **CS0770**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Nucleotide sugars, which are synthesized in the cytosol, are transported to the Golgi apparatus or ER compartments. The sugar residues are used by enzymes involved in protein glycosylation (yeasts and mammals) or polysaccharide synthesis (plants). The remaining NDPs (GDP or UDP) are degraded to NMPs by nucleoside diphosphatase (GDPase/UDPase). The NMPs are then used in antiport transport of new nucleotide sugars into the organelle lumen. Since GDPases/UDPases in yeasts are located exclusively in the Golgi complex, they are convenient markers for Golgi detection in yeast. ADPases are known to be expressed on the cell plasma membrane (endothelial cell and vascular precursors), where they participate in the nucleotide metabolism.

This kit provides a simple and reliable method for measuring NDPase activity in sub-cellular fractions. The method is based on the detection of the inorganic phosphate released from NDPs by the appropriate NDPase. The production of the inorganic phosphate is measured by a colorimetric reaction in the presence of molybdate ions. The color formation is proportional to the amount of the inorganic phosphate released by the enzymes and can be measured at 700 nm.

The kit was tested on rat tissue extracts (liver, kidney, spleen, and heart) and on a yeast (*S. cerevisae*) extract. It was also tested with microsomal fractions prepared from rat tissues and cell lines.

Components

The kit is sufficient for 300 assays in 96 well plates.

GDPase Assay Reagent Product Code G4920	10 ml
UDPase Assay Reagent Product Code U6008	10 ml
ADPase Assay Reagent Product Code A1730	10 ml
Sodium Dodecyl Sulfate Solution (SDS), 10% Product Code L4522	5 ml
Molybdenum Color Reagent Product Code M4944	60 ml
Ascorbic Acid Solution Product Code A1855	1 ml
Phosphorus Standard Solution Product Code P3869	1.7 ml

Reagents and Equipment Required but Not Provided

- Microplate reader
- 96 well plates flat bottom (Product Code Z712825 or equivalent)
- Ulta pure water

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.

Preparation Instructions

Defrost the kit components and mix until homogenous. Vortex the NDPase Assay Reagents for 10 seconds before use.

Molybdenum Reagent Working Solution - For one assay in a single well, mix 200 µl of Molybdenum Color Reagent (Product Code M4944) with 2 µl of Ascorbic Acid Solution (Product Code A1855). For assays of multiple samples calculate volumes accordingly. The Molybdenum Reagent Working Solution is stable for a few hours at room temperature.

Storage/Stability
The kit is shipped on dry ice and storage at –20 °C is recommended. After the first use, the 10% Sodium Dodecyl Sulfate Solution (Product Code L4522) should be stored at room temperature and the Phosphorus Standard Solution (Product Code P3869) and Molybdenum Color Reagent (Product Code M4944) should be stored at 2–8 °C.

Procedure
Determine the amount of total protein in the sample. The optimal amount of protein for an assay depends on NDPase activity of the sample. It is recommended to start with 15-20 µg of protein per assay. Ensure the samples do not contain reagents such as SDS or phosphate that may interfere with the assay. Samples should not be prepared with phosphate containing buffers.

Notes:
- Use ultrapure water.
- 10% SDS Solution (Product Code L4522) may precipitate at temperatures lower than 24 °C. Warm the SDS solution for 2-3 minutes at 37 °C before use.
- After defrosting the NDPase Assay Reagents, vortex thoroughly to dissolve any precipitate that may be formed during freezing.

The reaction mixtures for 96 well plate assays are described in Table 1.

Table 1.
Reaction Mixtures for 96 well plate assays

	Reaction Mixture			
	Sample	10% SDS Solution	Water	NDPase Assay Reagent
Test	30 µl	–	–	30 µl
Blank	30 µl	10 µl	–	30 µl
Standard	30 µl of Standard Solution	–	30 µl	–

1. Set the plate reader at 700 nm.

2. Add a protein sample containing 15-20 µg of total protein to the “Test” and “Blank” wells as detailed in Table 1. The sample volume should be 30 µl. For samples with a smaller volume, add water to bring the final volume to 30 µl.

3. Add 10 µl of the 10% SDS Solution (Product Code L4522) to the “Blank” well(s).

4. For determination of specific activity, include a “Standard” assay control reaction. Add 30 µl of the Phosphorus Standard Solution to a well. Add 30 µl of water to the same well. Mix gently by finger tapping.

5. Add 30 µl of NDPase Assay Reagent (ADPase, GDPase, or UDPase Assay Reagent as appropriate) to the “Test” and Blank” wells and mix gently by finger tapping. Do not add NDPase Assay Reagent to the Standard well(s). Incubate for 20 minutes at room temperature.

Table 2.
Reaction Termination and Phosphate Detection

	Stop Solution and Detection Solution	
	10% SDS Solution*	Molybdenum Reagent Working Solution
Test	10 µl	200 µl
Blank	–	200 µl
Standard	10 µl	200 µl

* Added in order to stop the reaction

6. Add 10 µl of SDS 10% solution (Product Code L4522) to the “Test” and “Standard” wells as detailed in Table 2. Mix gently by finger tapping. The SDS stops the reaction.
7. Add to all the wells 200 µl of Molybdenum Reagent Working Solution (see preparation instructions). Incubate for 10 minutes to allow color development.
8. Measure the absorption at 700 nm.
   Note: If the absorption at 700 nm is >1 or the sample is cloudy, reduce the amount of protein used in the assay.

Calculation

Calculate the NDPase specific activity.

Unit definition: 1 unit of NDPase releases 1 µmole of inorganic phosphate per minute at 25 °C under conditions specified by the assay.

$$\text{mU/mg} = \frac{(A_{700}\ \text{sample} - A_{700}\ \text{blank}) \times 19.5}{\text{time (min)} \times A_{700}\ \text{standard} \times \text{protein}}$$

mU/mg – specific activity (milliunits per mg of protein)
A_{700} sample – Absorbance of the "Test" sample
A_{700} blank – Absorbance of the "Blank" sample (sample in which SDS was added before the reaction was started)
A_{700} standard – The absorbance of the "Standard" sample
time – time in minutes of the incubation at room temperature
protein – amount of the protein (mg) added to the reaction
19.5 – nmoles of inorganic phosphate in 30 µl of the Phosphorus Standard Solution

References

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4. Gao, X.D., *et al*., A homologue of GDA1, encodes Membrane-bound apyrase required for Golgi N- and O-Glycosylation in *Saccharomyces cerevisiae*. J. Biol. Chem., **274**, 21450-21456 (1999).
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