Pyrophosphatase Assay Kit (BA0224)

Product Datasheet | SKU BA0224

Description

Inorganic pyrophosphatase (EC 3.6.1.1) catalyses the hydrolysis of phosphoester bonds on inorganic pyrophosphate, thereby releasing two orthophosphate molecules. Family I pyrophosphatases are essential enzymes found in all kingdoms of life and are responsible for maintaining the correct pyrophosphate equilibrium necessary to carry out nucleic acid and protein synthesis and to facilitate fatty acid beta-oxidation. This assay is based on a proprietary phosphate detection chemistry; the colour intensity, measured at 620 nm, is proportionate to the amount of phosphate released from pyrophosphate hydrolysis.

Specifications

Detection Method	Colorimetric; read at 600-660 nm (peak 620 nm)
Detection Range	1.0 to 20 U/L pyrophosphatase activity
Sample Type	Purified enzyme samples; biological samples
Species Reactivity	All
Assay Time	Approximately 60 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	Store all components at 4 degrees C upon receiving.
Shelf Life	6 months after receipt
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
Assay Buffer (pH 7.2)	8 mL	4 degrees C
Pyrophosphate	100 uL	4 degrees C
Standard (1 mM Phosphate)	120 uL	4 degrees C
POMG Reagent A	2.5 mL	4 degrees C
POMG Reagent B	120 uL	4 degrees C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0224	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Avoid phosphate-containing detergents and buffers when preparing pyrophosphatase samples as the assay is extremely sensitive to free phosphate; if the OD620nm for enzyme alone with the colour reagent is higher than 0.2, remove free phosphate by at least 3 washes with a 10 kDa NMWL membrane filter. Avoid samples with EDTA and metals such as Zn, Co, Mn and Ca, note that high protein concentrations can interfere through precipitation, and run a serial dilution in water for enzymes of unknown activity.
2. Enzyme reaction: pipette 10 microlitres of pyrophosphatase sample into separate wells of a clear flat-bottom 96-well plate, reserving one well per sample as a blank (10 microlitres of buffer, no enzyme).
3. Prepare enough Working Reagent by mixing 1 microlitre of pyrophosphate with 80 microlitres of Assay Buffer per assay well, initiate the reaction by adding 70 microlitres of Working Reagent into each well, tap the plate briefly to mix and incubate at room temperature (or the desired temperature) for 30 minutes.

4. Phosphate determination: prepare a 40 uM phosphate premix by pipetting 20 microlitres of the 1 mM phosphate standard into 480 microlitres of water, dilute the standards as shown in the standard preparation table and pipette 80 microlitres of each standard in duplicate into separate wells of the assay plate.
5. Prepare Colour Development Reagent by mixing 100 volumes of POMG Reagent A with 1 volume of POMG Reagent B (each well requires 20 microlitres); prepared reagent is stable for at least 1 day at room temperature.
6. At 30 minutes in the enzyme reaction step, add 20 microlitres of Colour Development Reagent to each well, mix gently by tapping the plate and incubate for a further 30 minutes at room temperature for colour development.
7. Measure absorbance at 600-660 nm (peak 620 nm) with a plate reader. Note: if the observed phosphate concentration is equal to or greater than the 40 uM Phosphate Standard, dilute the enzyme extract in buffer and repeat the assay.

Phosphate Standard Preparation

No	Premix + H2O	Final Vol (uL)	[Phosphate] (uM)
1	200 uL + 0 uL	200	40
2	120 uL + 80 uL	200	24
3	60 uL + 140 uL	200	12
4	0 uL + 200 uL	200	0

Calculation

Sample pyrophosphatase activity is calculated as: Enzyme Activity = [(OD_SAMPLE - OD_BLANK) / Slope] x [Reaction Vol (L) / (t (min) x Sample Vol (L))] x n (U/L) = [(OD_SAMPLE - OD_BLANK) / Slope] x 0.267 x n (U/L), where OD_SAMPLE and OD_BLANK are the sample and blank absorbances, Slope is the slope (uM-1) of the phosphate standard curve, Reaction vol and Sample vol are 80 uL and 10 uL respectively, t is the reaction time (30 min) and n is the sample dilution factor. Unit definition: one unit (U) of enzyme catalyses the production of 1 micromole of orthophosphate per minute under the assay conditions (pH 7.2).

Notes

- The assay is extremely sensitive to free phosphate; avoid phosphate-containing detergents and buffers, and remove free phosphate using a 10 kDa NMWL membrane filter if background is high.
- Avoid samples with EDTA and metals such as Zn, Co, Mn and Ca, and note that high protein concentrations can interfere with colour development through precipitation.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.