# Pyrophosphatase, Inorganic, GMP-Grade

# Catalog #GMP-PYR-YE101

**Storage Condition**  $-20^{\circ}C \pm 5^{\circ}C$  for 24 months. Avoid repeated freeze/thaw cycles.

Form Liquid

**Source** An *E. coli* strain that carries the gene for pyrophosphatase, Inorganic from *Saccharomyces cerevisiae* 

Storage Buffer 20 mM Tris-HCl, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, and 50% Glycerol, pH 8.0

Concentration 0.1U/µL

**Unit Definition** One unit is defined as the amount of enzyme that will generate 1µmol of orthophosphate per minute from inorganic pyrophosphate (PPi) in a 10 min standard reaction at 25°C containing 20 mM Tris-HCl, pH 8.0, 2 mM MgCl2, and 2 mM PPi.

# **Product Contents**

• Pyrophosphatase, Inorganic (0.1U/µL)

# **Product Description**

Pyrophosphatase, Inorganic catalyzes the hydrolysis of inorganic pyrophosphate to form two orthophosphates. Pyrophosphatase, Inorganic enhances RNA or DNA synthesis by preventing the accumulation of byproduct PPi generated in the reaction.

# Applications

- Improving RNA yield in In vitro transcription (IVT)
- Enhancing DNA amplification reaction
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#### **Quality Control Statement**

This product has been filed with the FDA Drug Master Files and is assigned DMF # 038030. KACTUS manufactures this product according to GMP guidelines and performs stringent quality control testing before release. The production is antibiotic- and animal-free.

# **Quality Control Release Criteria**

Assay	Criteria
Activity (Enzyme Catalytic Efficiency)	≥ 126U/mL
Purity (SEC-HPLC)	≥ 95%
Residual Endonuclease	Negative
Residual Exonuclease	Negative
Endotoxin	≤ 10EU/mL
Residual RNase	Negative
Residual DNase	Negative
Residual Protease	Negative
Residual Host Cell Protein	≤ 20ng/mg

Residual Host Cell DNA	≤ 100pg/mL
Bioburden	≤ 1CFU/10mL

# Protocol for In Vitro Transcription

1. Prepare the following reaction mixture:

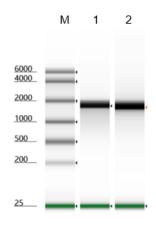
Reagent	Quantity
5X Transcription Buffer-1 (included with <u>T7 RNA Polymerase</u> )	4µL
CTP/GTP/ATP/UTP (100mM each)	2µL each
Murine RNase Inhibitor	1µL
Pyrophosphatase, Inorganic	1µL
T7 RNA Polymerase	2µL
Template DNA	1µg
RNase-free Water	Up to 20µL

- 2. Incubate at 37°C for 1-2 hours.
- 3. After transcription, add 2U <u>DNase I</u> to digest DNA template for 15 minutes at 37°C.
- 4. Inactivate DNase I by phenol/chloroform extraction.

#### Notes

- Pyrophosphatase, Inorganic works well in any buffer containing Mg<sup>2+</sup> (1-10 mM)
- The hydrolysis of PPi is a proportional to the concentration of enzyme.

# **Performance Validation**



The addition of Pyrophosphatase, Inorganic to IVT reactions improves mRNA yield. Lane 1: Without Pyrophosphatase, Inorganic; Lane 2: With Pyrophosphatase, Inorganic.