Pyrophosphatase, Inorganic (yeast, 0.1 U/μl, GMP Grade)

GMP4103PC

Animal-free, Ampicillin-free

Vazyme

Instruction for Use

Version 24.1

Contents

For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

Pyrophosphatase, Inorganic (inorganic pyrophosphatase) is an enzyme encoded by yeast DNA expressed in the recombinant *E. coli*, which can catalyze the hydrolysis of inorganic pyrophosphate into orthophosphate: $P_2O_7^{4-} + H_2O \rightarrow 2\ HPO_4^{2-}$. Under natural conditions, inorganic pyrophosphatases provide thermodynamic power for the biosynthesis of proteins, DNA, and RNA, and play a role in biotransformation processes such as lipid metabolism, calcium absorption, and bone formation. In nucleic acid amplification tests, this product can hydrolyze inorganic pyrophosphate generated during the reaction to prevent its inhibition against the reaction and promote the translation of the reaction equilibrium towards product generation.

This product is GMP grade recombinant Pyrophosphatase, Inorganic (yeast), produced with strict control of host protein, exogenous DNA, RNase and other process-related impurities, as well as microbiological limits and bacterial endotoxins are strictly controlled during the production process. The entire production process does not use or add ampicillin or any raw materials and auxiliary materials of animal origin, and adopts the production and quality management standards in accordance with the GMP norms to ensure that the production process and raw and auxiliary materials can be used in the production process. The production process and raw and auxiliary materials are traceable, and the product meets the requirements for raw and auxiliary materials in the field of mRNA vaccine production.

02/Product Components

Product Number	**GMP4103PC-01**	**GMP4103PC-02**	**GMP4103PC-03**
Product Specification	1 ml	5 ml	20 ml

03/Storage Conditions

Store at -20 ± 5℃ and transport at ≤0℃.

04/Product Information

Product Name	Pyrophosphatase, Inorganic (yeast, 0.1 U/μl)
Source	Recombinant *E. coli*
Activity	0.1 U/μl
Unit Definition	Under standard reaction conditions, one unit is defined as the amount of enzyme required to catalyze the hydrolysis of pyrophosphate (PPi) per minute to produce 1 μmol of orthophosphate (Pi)
Optimum Temperature	The optimal reaction temperature is 25°C and is active at 16 ~ 37°C
Cofactor	Mg^{2+}
Storage Buffer	20 mM Tris-HCl (25°C, pH 8.0), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol
Storage Conditions	-20 ± 5°C, avoid repeated freezing and thawing

05/Application

1. Optimize nucleic acid amplification reactions, improve the reaction efficiency and increase the nucleic acid yield.
2. Promote the synthesis of proteins and nucleic acids.
3. Used in other applications in the presence of PPi interference. Catalyze the degradation of PPi to eliminate its interference with the reaction.

06/Quality Standards

Items	Inspection Methods	Standards
Characteristics	Visual Inspection Method	Clear liquid
pH value	Ch.P2020 Part IV General rule 0631 pH value determination method	6.5 ~ 7.5
Activity	In vitro transcription fluorescence method	≥40 U/μl
Purity	Ch.P2020 Part IV General Regulation 0512 High performance liquid chromatography method	≥95%
DNA endonuclease	0.5 U of the product incubated with pUC19 plasmid at 37℃ for 4 h	Degradation ≤10%
DNA exonuclease	0.5 U of the product incubated with DNA substrate at 37℃ for 16 h	Degradation ≤10%
RNase	RNase fluorescence detection kit method	Negative
Exogenous DNA	Ch.P2020 Part IV General rule 3407 Exogenous DNA residue determination method third method quantitative PCR method	<120 fg/μl
Host Protein	Ch.P2020 Part IV General Regulation 3412 Method for the determination of protein residues in Escherichia coli organisms	<50 ppm
Heavy metal residues	Ch.P2020 Part IV General Regulation 0821 Heavy Metals Test Method No.1	≤10 ppm
Bacterial endotoxins	Ch.P2020 Part IV General Regulation 1143 Bacterial endotoxin test method 2 Photometric method	<2 EU/ml
Microbiological limits	Ch.P2020 Part IV General Regulation 1105	The total number of aerobic bacteria should be ≤5 cfu/ml
	Microbiological limit checks on non-sterile products: microbiological enumeration method	The total number of moulds and yeasts should be ≤5 cfu/ml

07/Experiment Procedures

1. Thaw all the kit components on ice, mix and pulse-spin in microfuge to collect solutions to bottom of tubes. Keep on ice.
2. Formulate a suitable reaction system according to the technical route:

2.1 In vitro transcription

Item	Recommended system	System scope adjustment	Final concentration
10 × Transcription Buffer (GMP Grade)	2 μl	2 μl	1 ×
T7 RNA Polymerase (200 U/μl, GMP Grade)	2 μl	0.5 - 3 μl	5 - 30 U/μl
Pyrophosphatase, Inorganic (yeast, 0.1 U/μl, GMP Grade)	1 μl	0 - 1 μl	0 - 5 mU/μl
Murine RNase Inhibitor (40 U/μl, GMP Grade)	1 μl	0 - 1 μl	0 - 2 U/μl
ATP/CTP/GTP/UTP Solution (100 mM)	Each 2 μl	Each 1 - 2 μl	Each 5 - 10 mM
Template DNA	1 μg	0.5 - 2 μg	25 - 100 ng/μl
RNase-free ddH_2O	Up to 20 μl	Up to 20 μl	

2.2 In vitro co-transcription

Item	Recommended system	System scope adjustment	Final concentration
10 × Transcription Buffer (GMP Grade)	2 μl	2 μl	1 ×
T7 RNA Polymerase (200 U/μl, GMP Grade)	2 μl	1 - 3 μl	10 - 30 U/μl
Pyrophosphatase, Inorganic (yeast, 0.1 U/μl, GMP Grade)	1 μl	0 - 1 μl	0 - 5 mU/μl
Murine RNase Inhibitor (40 U/μl, GMP Grade)	1 μl	0 - 1 μl	0 - 2 U/μl
CAG Trimer (100 mM, GMP Grade)	1.2 μl	0.5 - 1.8 μl	2.5 - 9 mM
ATP/CTP/GTP/UTP Solution (100 mM)	Each 1.5 μl	Each 1 - 2 μl	Each 5 - 10 mM
Template DNA	1 μg	0.5 - 2 μg	25 - 100 ng/μl
RNase-free ddH_2O	Up to 20 μl	Up to 20 μl	-

3. After thorough mixing, reaction conditions: 37°C, 2 h.
4. Add 1 μl of DNase I (1 U/μl, GMP Grade) (Vazyme #GMP4104PC) to the reaction system, mix well and centrifuge,react at 37°C for 15 min to degrade template DNA.
5. The synthesised RNA can be used for subsequent experiments or processes after purification and quality control.

08/Notes

1. In vitro transcription

1.1 The recommended system is suitable for initial experiments with new sequences, as there are several-fold or even 10-fold differences in the reaction rates of different sequences.

1.2 It is recommended to set up a T7 RNA Polymerase dosage gradient under the recommended system to confirm the appropriate dosage for specific incubation conditions.

1.3 The amount of NTPs determines the yield plateau of the system. Under the conditions of balanced ratio of the four bases and sufficient amount of T7 RNA Polymerase, the yield plateau of each 10 mM (final concentration) amount of NTPs is 180 - 240 μg/20 μl.

1.4 All enzyme products contain glycerol, it is recommended that the total volume of enzyme products added to the system should not exceed 1/5 of the reaction volume, and it is recommended that the repeated freezing and thawing should not be more than 7 times in use.

1.5 Template DNA can be obtained by post fermentation linearisation or PCR amplification; RNase A residues introduced during plasmid DNA extraction can significantly affect the quality of the transcribed RNA, and it is recommended to use a high purity RNase-free plasmid with an OD260/280 of 1.8 - 2.0.

1.6 The yield is directly proportional to the reaction time. In cases where the recommended reaction conditions do not achieve the desired yield, extending the reaction time can be chosen to reach the target yield plateau. The reaction time can be adjusted within a range of 0-4 hours based on specific requirements.

1.7 Natural RNA can be quantified using a UV spectrophotometer; some modified nucleosides affect UV absorbed light resulting in quantitation results that differ from those of natural nucleosides, which can be more accurately quantified using the fluorescence detection reagent Equalbit RNA BR Assay Kit (Vazyme #EQ212).

1.8 Product-related impurities dsRNA can be quantified using the dsRNA (Modified) Quantification Kit (ELISA) 2.0 (Vazyme #DD3509).

1.9 This in vitro transcription assay procedure yields uncapped RNA with a 5' triphosphate structure that cannot mediate eukaryotic translation. To obtain mRNA with Cap1 structure, use Vaccinia Capping Enzyme (10 U/μl, GMP Grade) (Vazyme #GMP4109PC) and mRNA Cap 2'-O-Methyltransferase (50 U/μl, GMP Grade) (Vazyme #GMP4110PC) for in vitro capping; Cap1 mRNA can also be obtained in one step by referring to "2.2 In vitro co-transcription".

2. In vitro co-transcription

2.1 The in vitro co-transcriptional initiation sequence needs to match the base type of the cap analog, generally using “AG” as the transcription initiation sequence to achieve higher capping efficiency, paired with the corresponding natural cap analog CAG Trimer (100 mM, GMP Grade) (Vazyme #GMP4118PC).

2.2 Due to differences in transcription initiation mechanisms, the in vitro co-transcriptional rate is generally 1/2 - 1/5 of the in vitro transcription rate.The recommended in vitro co-transcriptional system can be followed to achieve the desired reaction rate.

2.3 The amount of cap analog input for the recommended system usually yields mRNAs with >90% cap rate.

2.4 For the rest of the precautions, see "1. In vitro transcription".

09/Related Products

Product Number	Product Name
GMP4101R	10 × Transcription Buffer (GMP Grade)
GMP4101PB	T7 RNA Polymerase (200 U/μl, GMP Grade)
GMP4103PC	Pyrophosphatase, Inorganic (yeast, 0.1 U/μl, GMP Grade)
GMP4104PC	10 × DNase I Reaction Buffer (GMP Grade)
GMP4102PA	Murine RNase Inhibitor (40 U/μl, GMP Grade)
GMP4109PC	Vaccinia Capping Enzyme (10 U/μl, GMP Grade)
GMP4110PC	mRNA Cap 2'-O-Methyltransferase (50 U/μl, GMP Grade)
GMP4118PC	CAG Trimer (100 mM, GMP Grade)
DD3509	dsRNA (Modified) Quantification Kit (ELISA) 2.0
EQ212	Equalbit RNA BR Assay Kit

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