Murine RNase Inhibitor (40U/μl, GMP Grade)

GMP4102PA

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

Murine RNase Inhibitor is a recombinant *E. coli*-expressed murine RNase inhibitor that binds RNase A, B, and C 1:1 in a non-competitive manner to inhibit the activity of all three enzymes and protect RNA from degradation. It has good thermal stability and is compatible with various commercially available reverse transcriptases, DNA and RNA polymerases, and capsulases. Compared with Human RNase Inhibitor, Mouse RNase Inhibitor does not contain the two cysteines in the human protein that are very sensitive to oxidation, thus it has higher antioxidant activity, and is more suitable for experiments that are sensitive to high reducing agents, such as qPCR, etc.

This product is a GMP-grade recombinant Murine RNase Inhibitor. During the production process, host protein, exogenous DNA, RNase and other process-related impurities, as well as microbiological limits and bacterial endotoxins are strictly controlled. 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 GMP norms to guarantee the traceability of the production process and raw and auxiliary materials, and the product meets the requirements for raw and auxiliary materials in the field of mRNA vaccine production and other fields.

02/Product Components

Product Number	**GMP4102PA-01**	**GMP4102PA-02**	**GMP4102PA-03**
Product Specification	1 ml	5 ml	20 ml

* Associated Product: 10 × DNase I Reaction Buffer (GMP Grade) (Vazyme #GMP4104R)

03/Storage Conditions

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

04/Product Information

Product Name	Murine RNase Inhibitor (40 U/μl, GMP Grade)
Source	Recombinant *E. coli*
Activity	40 U/μl
Unit Definition	The amount of enzyme required to inhibit 5 ng of RNase A activity by 50% is defined as 1 unit of activity (RNase A activity was determined by inhibiting hydrolysis of Cyclic 2',3'-CMP to produce 3'-CMP)
Optimum Temperature	Active at 25 ~ 55°C
Optimum pH	It showed maximum activity at pH 7.0 - 8.0 and was activity at pH 5.0 - 9.0.
Storage Buffer	20 mM Hepes, 50 mM KCl, 5 mM DTT, 50% glycerol, pH 8.0
Storage Conditions	-20 ± 5°C, avoid repeated freezing and thawing

05/Application

Suitable for experiments that require RNase removal and are sensitive to high reducing agents, such as qPCR.

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	7.5 - 8.5
Activity	Fluorescence method	≥40 U/μl
Purity	Ch.P2020 Part IV General Regulation 0512 High performance liquid chromatography method	≥95%
DNA endonuclease	200 U of the product incubated with the pUC19 plasmid at 37°C for 4 h	Degradation ≤10%
DNA exonuclease	200 U of the product incubated with the DNA substrate for 16 h at 37°C	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	≤10 ppm
Heavy metal residues	Ch.P2020 Part IV General Regulation 0821 Heavy Metals Test Method No.1	≤10 ppm
Mycoplasma	Mycoplasma Detection Kit Method	<2 EU/ml
Bacterial endotoxins	Ch.P2020 Part IV General Regulation 1143 Bacterial endotoxin method 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 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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