KRISHGEN BioSystems
OUR REAGENTS, YOUR RESEARCH

KRISHZYME™ T7 RNA Polymerase

Catalog Number: KLPL10301 / KLPL10302 / KLP10303

Description

Krishzyme™ T7 RNA Polymerase is a DNA-dependent RNA polymerase from T7 phage that possesses a strong and specific 5´→ 3´ RNA polymerase activity. It has a high specificity for T7 promoter sequences and will synthesize large quantities of RNA from a DNA fragment inserted downstream from a promoter.

Expression Host:

E.coli

Purity:

>98% as determined by SDS-PAGE quantitative densitometry by Coomassie Blue Staining.

Concentration:

It is supplied at a concentration of 50 U/ul along with 10X HH T7 Buffer for reconstitution.

Unit Definition:

One unit is defined as the amount of enzyme required to incorporate 1 nmol ATP into an acid-insoluble material in 1 hour at 37°C.

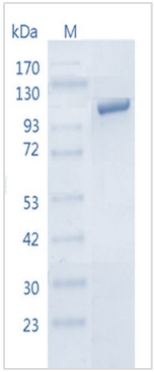
Formulation:

KRISHZYME™ T7 RNA Polymerase is supplied as a lyophilized powder.

Molecular Mass:

The KRISHZYME™ Recombinant Trypsin has a calculated molecular mass of ~24 kDa

SDS-PAGE:
Fig.1.



KDa Marker 10-170

Fig. 1. Purity analysis by SDS-PAGE Detection

Reconstitution:

Being an enzyme, the concentration may differ from lot to lot. We always recommend referring the accompanying data sheet to view the exact concentration and the recommended dilution schemata.

Centrifuge the vial at 4ºC before opening to recover the entire contents. Please contact us for any concerns or special requirements at +91-22-49198700 | Email: sales1@krishgen.com

Storage:

Store it under sterile conditions at -20ºC to -80ºC upon receiving for at least 12 months. It is recommended to aliquot the enzyme into smaller quantities for optimal storage. Avoid repeated freeze-thaw cycles.

Product Instruction:

Long-term storage: If the prepared recombinant cell digestion solution needs to be stored for a longer period, Store at -20°C not exceeding 12 months from date of preparation.

RNA Synthesis Reaction:

Reagent	Amount
Nuclease-free water	Up to 20 ul
10×HH T7 Buffer	2 ul
ATP/GTP/CTP/UTP (10 mM each)	2 ul each (1 mM each Final)
RNase Inhibitor (40 U/ul)	1 ul
Pyrophosphatase Inorganic (0.1 U/ul)	2 ul
T7 RNA Polymerase (50 U/ul)	2 ul
Linearized DNA Template	1 ug

Incubation Time:
37ºC for 1-2 hours.

Stop of Reaction:
Add 2 ul of 0.2 M EDTA (pH=8.0@25ºC) or heat to 75°C for 10min.

DNA Removal
DNA template can be removed with 2U DNase I (RNase-free) and incubation for 15 min at 37°C.

Inhibitors
Metal chelators, enzyme activity is reduced by 50% at NaCl or KCl concentration above 150 mM.

Precaution

- *The transcription reaction should be performed under contamination without RNases. Wearing gloves is advisable. The tips, tubes and water should be nuclease free. All the solutions should be made up in nuclease free water.*
- *The RNA synthesis reaction mixture should be prepared at room temperature, since DNA may precipitate in the presence of spermidine at 4°C.*
- *The yield of proper length transcripts decreases if the template DNA is incompletely linearized.*
- *The reaction mixture can be scaled up or down.*

Quality Control Assay:

- Endonuclease Activity:
  Incubation of a 40 ul reaction containing a minimum of 200 U of T7 RNA Polymerase with 4 ug pUC19 DNA for 16 hours at 37ºC results in no detectable degradation as determined.

- Exonuclease Activity:
  Incubation of a 50 ul reaction containing a minimum of 200 U of T7 RNA Polymerase with 1 ug Hind III digest λ DNA for 16 hours at 37ºC results in no detectable degradation as determined.
- Nickase Activity:
  Incubation of a 50 ul reaction containing a minimum of 200 U of T7 RNA Polymerase with 1 ug pBR322 DNA for 16 hours at 37ºC results in no detectable degradation as determined.
- RNase Activity:
  Incubation of a 50 ul reaction containing a minimum of 200 U of T7 RNA Polymerase with 1.6 ug MS2 RNA for 4 hours at 37ºC results in no detectable degradation as determined.
- Heat Inactivation: 75ºC for 10min.

Application:

In Vitro Transcription (IVT)
- Synthesis of RNA from DNA templates bearing the T7 promoter.
- Used to produce:
  - mRNA for research or therapeutic purposes
  - Riboprobes (labeled RNA probes for hybridization)
  - Guide RNAs for CRISPR-Cas9 systems

- mRNA Vaccine Production
- Core enzyme in the production of synthetic mRNA vaccines
- Used with modified nucleotides like N1-methyl-pseudouridine to improve mRNA stability and reduce immunogenicity.

- RNA Structure and Function Studies
- Generation of long RNA transcripts for:
  - Structural studies (e.g., NMR, cryo-EM)
  - RNA folding analysis
  - RNA-protein interaction assays

- RNA Probe Generation
- Radioactively or fluorescently labeled RNA probes for:
  - Northern blotting
  - In situ hybridization (ISH)
  - Microarrays

- Aptamer and Riboswitch Research
- In vitro transcription of RNA libraries for:
  - SELEX (Systematic Evolution of Ligands by Exponential Enrichment)
  - Riboswitch discovery

- Run-Off Transcription for Functional Assays
  To study:
  - Transcription kinetics
  - Polymerase activity under different conditions
  - RNA processing events like capping and polyadenylation

- Synthetic Biology & Cell-Free Systems
- Used in cell-free protein expression systems such as:
  - PURE (Protein synthesis Using Recombinant Elements) system
  - TX-TL (Transcription-Translation) platforms

References:

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