Imagen ® Reverse Transcriptase MIIIs

Cat. NO IPQ

Description

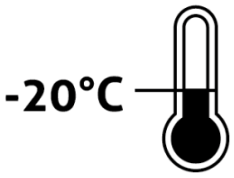
Imagen ® Reverse Transcriptase MIIIs is a genetically engineered multiple-point mutanted version of M-MLV RT. The enzyme is purified from E. coli containing the mutanted pol. gene of Moloney Murine Leukemia Virus . The enzyme can be reverse first-strand cDNA at higher temperatures M-MLV RT , providing fast、high specificity、yield of cDNA. The cDNA obtained with this product can be used in both intercalator qPCR assay or probe qPCR assay or cDNA library construction and amplification of some PCR targets.

Contents

The **Imagen ® Reverse Transcriptase MIIIs** contains Reverse Transcriptase MIIIs, 5X First-Strand Buffer(DTT、dNTPs), Storage Buffer(Tris-HCl PH7.8、NaCl、EDTA、DTT、Glycerol).

Storage

- ✓ -20 °C
- ✓ Avoid repeated freezing and throwing



Reaction Mix Thawing and Handling

To use the mix, thaw the vial on ice to 4 °C.

Please completely mix the vial and briefly centrifuge to ensure all components are at the bottom of the tube. Store on ice protected from light until ready to use. If using automated liquid handling, let sit at ambient temperature for 10 min to further reduce the viscosity.

Unit Definition

- ✓ One unit incorporates 1 nmole of dTTP into acid precipitable material in 10 mins at 37°C using poly(A)-oligo(dT) as template primer

Application

- ✓ RNA transcription to cDNA
- ✓ cDNA can be further applied to downstream such as PCR, qPCR and sequencing.

Prepare the RT-PCR Reaction Mix

1. Mix the **Imagen ® Reverse Transcriptase MIIIs** thoroughly but gently until it's completely homogenous.
2. Prepare the Reverse Transcription Reaction Mix for the number of reactions required as shown in table below.

Component	Volume (ul)
Oligo d(T)$_{18\text{-}20}$ primer 50 pmole / Random hexamers 50 pmole /Gene specific primer 2 pmole	1
5X First-Strand Buffer	4
Imagen ® Reverse Transcriptase MIIIs Premix	1
RNA Template(total RNA 10ng ≦ 2 µg or mRNA 10ng ≦ 1µg)	-
Nuclease-free water	-
Final volume	**20**

3. Add component to the PCR tubes or wells containing the reaction mix, seal tubes or wells with flat caps or optically transparent film, and gently vortex to ensure thorough mixing of the reaction components.
4. Program the thermal cycling protocol on the PCR instrument.

Step	Temp. °C	Time	Cycles
Reverse Transcription (1Kb↓10min ；1Kb↑30min)	48°C	10 -30 mins	1
Denature RTase	85°C	5 mins	1

6. Load the PCR tubes or plates onto the real-time PCR instrument and start the qPCR run program.
7. Store products at-20°C, and proceed to PCR amplification or qPCR using 2µl first-strand cDNA synthesis reaction mixture.

Prepare the PCR Reaction Mix

1. Add the following components to a PCR reaction tube:

Use only 2µl of the First-Strand reaction for PCR	
Component	Volume
10X PCR Buffer	5 µl
10 mM dNTPs mix	1 µl
10µM Forward primer	1 µl
10µM Reverse primer	1 µl
5U/µl Taq DNA polymerase	0.5 µl
The First-Strand reactant	2 µl
Autoclaved, distilled water	to 50 µl

2. Mix gently and spin down.
3. Perform PCR reaction according to the supplier instructions.

Prepare the qPCR Reaction Mix

1. Add the following components to a PCR reaction tube:

Use only 2µl of the First-Strand reaction for qPCR	
2X qPCR Premix	12.5 µl
10µM Forward primer	0.75 µl
10µM Reverse primer	0.75 µl
The First-Strand reactant	2 µl
Autoclaved, distilled water	To 25ul

2. Mix gently and spin down.
3. Perform qPCR reaction according to the supplier instructions.