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RealScript™ cDNA Synthesis Supermix

Description

RealScript™ cDNA Synthesis Supermix is specially designed for reverse transcription with any amount of RNA up to 5 µg per reaction. This optimized super mix contains all the factors needed for first-strand cDNA synthesis, including RealScript™ Reverse Transcriptase and 2X First-Strand Reaction Mix.

RealScript™ Reverse Transcriptase is an unique enzyme, different from the reverse transcriptases of Moloney Murine Leukemia Virus (MMLV) or Avian Myeloblastosis Virus (AMV). As a version of mutated MMLV, RealScript™ Reverse Transcriptase is genetically engineered to increase half-life, reduce RNase H activity, increase thermal stability, increase specificity of RT, provide more full-length product and lead to the highest cDNA yield of all RTs.

RealScript™ cDNA Synthesis Supermix is ideal for cDNA synthesis using a gene-specific primer, random primer, or either total RNA or poly(A)+-selected RNA primed with oligo(dT). By providing high yields of first-strand cDNA in a convenient high-throughput super mix format, RealScript™ cDNA Synthesis Supermix makes reverse transcriptase simple and easy.

Specifications

Cat. No.	Product Name	Specification
RR001	RealScript™ cDNA Synthesis Supermix, 50 reactions	RealScript™ Reverse Transcriptase (200U/µl):50µl 2X First-Strand Reaction Mix: 500µl
RR002	RealScript™ cDNA Synthesis Supermix, 100 reactions	RealScript™ Reverse Transcriptase (200U/µl):100µl 2X First-Strand Reaction Mix: 1000µl
RR001S	RealScript™ cDNA Synthesis Supermix, 10 reactions	RealScript™ Reverse Transcriptase (200U/µl): 10µl 2X First-Strand Reaction Mix: 100µl

Features

- Reduced RNase H activity results in more full-length cDNA.
- Half life of 100 minutes at 50℃ for the highest cDNA yields.
- Ability to increase RT units without inhibiting subsequent PCR.
- Full activity at 50℃ for increased specificity with gene-specific primers (GSP).

Content

- RealScript™ Reverse Transcriptase
- 2X First-Strand Reaction Mix Contains:
  100 mM Tris-HCl pH 8.3, 150 mM KCl, 6 mM $MgCl_2$, 20 mM DTT and 1 mM dNTPs.

Unit Definition

One unit incorporates 1 nmole of dTTP into acid precipitable material in 10 minutes at 37℃ using poly(A)-oligo(dT) as template primer.

Quality Control

RealScript™ cDNA Synthesis Supermix has passed the following quality control assays: SDS−polyacrylamide gel analysis for purity; functional absence of endodeoxyribonuclease, 3’ and 5’ exodeoxyribonuclease, and ribonuclease activities; yield and length of cDNA product.

Applications

- Synthesis of first-strand cDNA
- cDNA libraries
- Array labeling
- RT-PCR, primer extension, and 3' and 5' RACE

Shipping and Storage Conditions

RealScript™ cDNA Synthesis Supermix is shipped on dry ice and should be stored immediately upon receipt at -20℃ in a constant temperature freezer. With proper storage, RealScript™ cDNA Synthesis Supermix can be stored for up to 12 months without showing any deduction in performance and quality.

Notes

- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- This product is developed, designed and sold for research use only. Not for use in diagnostic or therapeutic procedures.

Protocol

Standard Protocol for First-Strand cDNA Synthesis (total reaction size is 20ul):

1. Add the following components to a sterile microtube on ice:

Component	Volume/ Reaction
2X First-Strand Reaction Mix	10 µl
Oligo (dT) primer	50 pmole
or Random primer	50 pmole
or Gene specific primer	2 pmole
Template RNA	total RNA ≤ 5 µg **or** mRNA ≤ 1 µg
Sterilized DDH_2O	Add to 18 µl

2. Incubate the mircrotube at 65℃ for 5 minutes.
3. Cool immediately on ice for 30 seconds and spin down.
4. Add the following components to the microtubes:

Component	Volume/ Reaction
RealScript™ Reverse Transcriptase (200U/µl)	1 µl
RNase Inhibitor (optional)	1 µl

5. Mix gently and spin down.
6. Incubate the mircrotube at 30℃ for 10 minutes. (optional for random primer)
7. Incubate the mircrotube at 42℃ for 30-60 minutes.
8. Incubate the mircrotube at 70℃ for 15 minutes.

Suggested PCR parameters (Use only 2 µl of the first-strand reaction for PCR):

1. Add the following components to a sterile PCR tube on ice.

Component	Volume/ Reaction
10X PCR Buffer	5 µl
10 mM dNTPs Mixture	1 µl
10 µM Forward primer	1 µl
10 µM Reverse primer	1 µl
5 U/µl Taq DNA polymerase	1 µl
The first-strand reactant	2 µl
Sterilized DDH_2O	Add to 50µl

2. Mix gently and spin down. Perform 20 to 40 cycles of PCR.