



FIREScript RT cDNA Synthesis MIX without primers

Cat. No.	20 µl Reactions
06-17-00100	100
06-17-00500	500

For *in vitro* use only.

Description:

FIREScript Reverse Transcriptase (RT) is a genetically engineered MMLV (*Moloney Murine Leukemia Virus*) based Reverse Transcriptase. This is an RNA-directed DNA polymerase that can synthesize a complementary DNA strand from ssRNA or ssDNA and is active over a broad range of reaction temperatures from 37°C-60°C. **FIREScript RT** is a robust enzyme for RNA detection and has enhanced stability at room temperature with no activity loss for up to 1 month. This RT contains a functional RNase H domain which can increase the sensitivity of RT-qPCR (quantitative reverse transcription PCR).

Source:

Purified from an *E.coli* strain that carries an overproducing plasmid containing a *FIREScript Reverse Transcriptase* gene.

Applications:

- First strand cDNA synthesis
- RT-PCR
- RT-qPCR

Reagents Provided:

- **FIREScript Enzyme Mix**
FIREScript RT and RiboGrip RNase inhibitor
- **10x RT Reaction Premix without primers**
RT reaction buffer with DTT and dNTPs
- **Water, nuclease free**

Unit definition:

One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into acid-precipitable material in 10 minutes at 37°C using poly(rA)•oligo(dT) as template in a total reaction volume of 50 µl.

FIREScript Storage and Dilution buffer:

50% glycerol (v/v), 20 mM Tris-HCl pH 7.5 at 25°C, 100 mM KCl, 0.1 mM EDTA and stabilizers.

Quality control:

Free of endo- and exodeoxyribonucleases, phosphatases and ribonucleases. Activity and stability tested in first strand cDNA synthesis. SDS/PAGE - 74 kD monomer, >98% pure.

Shipping and Storage conditions:

Routine storage: -20°C

The following conditions have no detrimental effects on the quality of the reagents:

- Shipping and temporary storage at room temperature for up to 1 month
- Storage at +4°C for up to 6 months
- 30 freeze-thaw cycles

General protocol recommendations:

For templates longer than 5 kb it is recommended to perform reverse transcription at 37°C for 30 min.

For templates with high secondary structure or gene specific primers with high melting temperature the reverse transcription temperature may be increased up to 60°C.

Recommended protocol:

Thaw and mix the following reaction components in a nuclease-free microcentrifuge tube.

Component	Volume	Final conc.
Template RNA	0,1 ng - 5 µg	variable
10x RT Reaction Premix without primers	2 µl	1x
Oligo(dT) primer (100 µM)/ random primers (100 µM)/ gene specific primer	1 µl	5 µM 5 µM 0,1-1 µM
FIREScript Enzyme Mix	1,5 µl	
Nuclease-free H ₂ O	Up to 20 µl	
Total	20 µl	

Use the following programme for cDNA synthesis:

Step	Temperature	Time
Primer annealing (<i>ONLY if using random primers</i>)	25°C	5-10 min
Reverse transcription	37-60°C	15-30 min
Enzyme inactivation	85°C	5 min

The volume of cDNA should not exceed 1/10 of the PCR or qPCR reaction volume.

Safety warnings and precautions:

This product and its components should be handled only by persons trained in laboratory techniques. It is advisable to wear suitable protective clothing, such as laboratory overalls, gloves and safety glasses. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

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