Solis BioDyne
Data Sheet

FIREScript KIT

Cat. No.	20 µl Reactions
06-13-0000S	20 rxn SAMPLE
06-13-00050	50
06-13-00200	200
06-13-01000	1000

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 Reverse Transcriptase** (200 U/µl)
- **10x RT Reaction Buffer with DTT**
  500 mM Tris-HCl pH 8.3, 500 mM KCl, 30 mM $MgCl_2$, 100 mM DTT

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 quick protocol:

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

Component	Volume	Final conc. (20 µl)
Template RNA	0,1 ng - 5 µg	variable
Oligo(dT) primer (100 µM)/ random primers (100 µM)/ gene specific primer	1 µl	5 µM 5 µM 0,1-1 µM
dNTP MIX (20 mM of each)	0,5 µl	500 µM
10x RT Reaction Buffer with DTT	2 µl	1x
FIREScript RT	1 µl	10 U/µl
RiboGrip RNase Inhibitor (40 U/µl)	0,5 µl	1 U/µl
Nuclease-free H_2O	Up to 20 µl	
Total	**20 µl**	

Use the following programme for cDNA synthesis:

Step	Temperature	Time
Primer annealing *(ONLY if using random primers)*	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.

Recommended standard protocol:

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

Component	Volume	Final conc. (20 µl)
Template RNA	0,1 ng - 5 µg	variable
Oligo(dT) primer (100 µM)/ random primers (100 µM)/ gene specific primer	1 µl	5 µM 5 µM 0,1-1 µM
Nuclease-free H_2O	Up to 16 µl	
Total	**16 µl**	

Incubate the template RNA and primer mix at 65°C for 5 min and then place on ice.

After a short spin add the following components.

Protocol continues →

Component	Volume	Final conc. (20 µl)
10x RT Reaction Buffer with DTT	2 µl	1x
dNTP MIX (20 mM of each)	0,5 µl	500 µM
FIREScript RT	1 µl	10 U/µl
RiboGrip RNase Inhibitor (40 U/µl)	0,5 µl	1 U/µl
Total	**20 µl**	

Use the following programme for cDNA synthesis:

Step	Temperature	Time
Primer annealing *(ONLY if using random primers)*	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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