DTI FabScript Reverse Transcriptase

Instruction Manual for use

User Manual for DTI FabScript Reverse Transcriptase

Catalogue number: DT0301.10K

Note: Applicable to all pack sizes

Manufactured by
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1) Product Description:

DTI FabScript Reverse Transcriptase is a modified M-MLV (Moloney Murine Leukemia Virus) RTase. This enzyme has extremely high extension capability and can synthesize long 1st-strand cDNA efficiently. Even for difficult templates, including RNAs with complex secondary structure, it is possible to synthesize 1st-strand cDNA at the normal reverse transcription temperature (42℃) with this enzyme. It is not necessary to perform the RT reaction at higher temperatures, a condition that may cause RNA degradation. This enzyme is suitable for preparation of long cDNAs, construction of cDNA libraries that include full-length cDNA, etc.

2) Concentration : 200 U/ul

3) Storage Buffer :

Tris-HCl (pH 7.8)	20 mM
NaCl	100 mM
EDTA	1 mM
DTT	1 mM
Glycerol	50%

4) Composition of Supplied Reagent :

5X FabScript Buffer (for cDNA synthesis)

250 mM	Tris-HCl, pH 8.3
375 mM	KCl
15 mM	$MgCl_2$

5) Storage : -20℃

6) Source:

E. coli carrying the plasmid that encodes reverse transcriptase gene.

7) Unit definition :

One unit is the amount of the enzyme that incorporates 1 nmol of dTTP in 10 minutes at 37°C, with poly (rA) · oligo $(dT)_{12\text{-}18}$ as the primer-template.

8) Quality Control Data

Please see the Certificate of Analysis (CoA) for each lot.

9) Applications :

- First-strand cDNA synthesis.
- Preparation of cDNA probes.
- RT-PCR.

10) Materials required but not provided

Reagents: dNTP, cDNA synthesis specific reverse primers/Oligo dT primer/Random Hexamers, PCR kit, sterile purified water, template

Equipment: Thermal cycler (DTI FabSpeed thermal cycler, model# TCST-9622)

Consumables: PCR tubes, Micropipettes and tips

11) Standard protocol for 1st-strand cDNA synthesis :

i. Prepare the following mixture in a microtube.

Oligo dT primer	50 pmol
(or random primer (6 mers)	50 pmol)
(or gene specific primer	2 pmol)
dNTP Mixture (10 mM each)	1 µl
Template RNA	total RNA ≦ 5 µg, mRNA ≦ 1 µg
RNase free dH2O	up to 10 µl

ii. Heat at 65°C for 5 min and cool immediately on ice.

iii. Prepare the reaction mixture by combining the following in a total volume of 20 µl.

Template RNA/Primer mixture	10 µl
5X FabScript Buffer	4 µl
RNase Inhibitor	20 U
DTI FabScript Reverse Transcriptase	100 - 200 U*1
RNase free dH2O	up to 20 µl

*1 100 U is recommended for RT-PCR and cDNA cloning;
200 U is recommended for quantitative analysis, such as qPCR.

iv. Mix gently.

v. Perform the reaction under the following condition.

30°C	10 min*1
42 (- 50)°C*2	30 - 60 min*3

*1 This step is required for random primer.

*2 It is generally recommended to perform the RT reaction at 42℃. However, for RT-PCR, if the reverse primer for PCR is also used as a RT primer, non-specific products may be amplified due to mispriming. In such a case, perform the RT reaction at 50℃ for 30 min.

*3 In most cases, 30 min is sufficient. Increase the incubation time to 60 min when the target is very long.

vi. Heat at 70°C for 15 min and cool on ice.

The obtained cDNA can be used for 2nd-strand cDNA synthesis or as a template for PCR.

12) Experimental sample:

Amplification of 6 kb and 12 kb fragments from rat brain total RNA was observed. The protocol used for the assay and the results are as follows

cDNA synthesis

42C	60 min
70C	15 min
9C	∞

PCR condition

94C	1 min	
98C	10 sec	30 Cycles
68C	12 min	
72C	10 min	

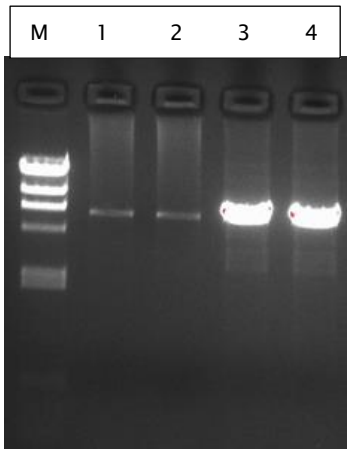

Figure 1A: Gel electrophoresis image of PCR for 6 kb fragment

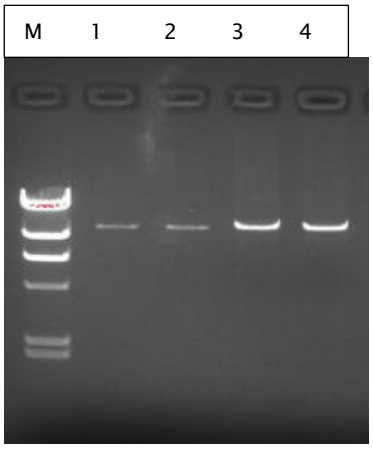

Figure 1B: Gel electrophoresis image of PCR for 12 kb fragment

Lane M: λ Hind III digest marker
Lane 1-2: Test – 10 ng
Lane 3-4: Test – 100 ng

Visit https://dsstakarabio.com/pages/dti-fabscript-reverse-transcriptase for more detailed product information

For more information contact directly below;
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Toll-Free number 1800-212-4922

Description of Symbol Used:

Catalogue number

Batch Code


Date of Manufacturing


Use-by-date


Contains sufficient for <n> tests