MINOTECH RT

Catalogue No **801-1(10KU)**

Concentration **200U/µl**

Store at -20°C.

Reagents supplied: 5x MINOTECH RT assay buffer

Source: Purified from an *E. coli* strain carrying a plasmid with M-MuLV reverse transcriptase gene.

Description: High purity reverse transcriptase suitable for first strand cDNA synthesis.

Unit definition: One unit is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into acid-insoluble material in a total reaction volume of 50 µl in 10 minutes at 37°C using poly(rA)•oligo(dT)18 as template.

Quality Control Assays:

- Functional Assay: MINOTECH RT is tested for performance in First strand cDNA synthesis followed by PCR with Taq polymerase. The resulting 1.600 bp PCR product is visualized as a single band on an ethidium bromide-stained agarose gel. *MINOTECH RT has been successfully used for synthesis of DNA fragments up to 8.8kb size.*
- Absence of contaminants: Tested extensively for the absence of nicking, endo- and exodeoxyribonucleases and RNases

Guaranteed stability: MINOTECH RT is guaranteed to maintain stability until expiration date

Recommended First-Strand cDNA Synthesis mixture:

- 1 µl of oligo(dT) 20 (50 µM); or 200–500 ng of oligo(dT) 12-18 ; or
  50–250 ng of random primers; or 2 pmol of gene-specific primer
- 10 pg–5 µg total RNA or 10 pg–500 ng mRNA
- 1 µl 10 mM dNTP Mix (10 mM each dATP, dGTP, dCTP and dTTP at neutral pH)
- Sterile ultrapure water up to 13 µl.

1. Heat mixture to 65°C for 5 minutes

2. Incubate on ice for at least 1 minute

3. Brief centrifugation and add:

- 4 µl 5X MINOTECH RT assay buffer
- 1 µl 0.1 M DTT
- 1 µl RNase Inhibitor (40 units/µl).
  1 µl of MINOTECH RT (~200 units/µl)*

* 400 U of MINOTECH RT can be added to increase yield (for the generation of cDNA >5kb).

4. Mix gently. If using random primers, incubate tube at 25°C for 5 minutes.

5. Incubate at 37-42°C for 30–60 minutes.

6. Heat inactivation step at 70°C for 15 minutes.

Optional (recommended for PCR targets >1kb). Remove RNA complementary to the cDNA, by adding 2 units *of E. coli* RNase H and incubate at 37°C for 20 minutes.

MINOTECH BIOTECHNOLOGY

Recommended PCR mixture:

10x MINOTECH Taq pol. buf.	5 µl
10mM dNTP mix	1 µl
25µM forward primer	1 µl
25µM reverse primer	1 µl
cDNA(from first-strand reaction)	2 µl
MINOTECH Taq DNA pol. (5 u/µl)	0.25-0.5 µl
Sterile ultrapure water	Up to 50 µl

Recommended PCR conditions:

Initial denaturation		94°C, 2min
25-35 PCR Cycles	Denature	94°C, 45sec
	Anneal*	45-68°C, 30sec
	Extend	72°C, 1min/kb
Final extension		72°C, 10min
Hold		4°C, indefinitely

**Anneal temperature depends on primer Tm*