SciPhi™

SciPhi™ M-MulV Reverse Transcriptase

Catalogue No.	Product Components	
	Reverse Transcriptase	5X RT buffer
NXG662-L	5x 10000U	5x 1 mL
NXG662-M	2x 10000U	2x 1 mL
NXG662-S	10000U	1 mL
NXG662-XS	2000 U	0.2 mL
Store kit and all components at -20°C		

Product Description

SciPhi™ Reverse Transcriptase is a genetically engineered version of M-MuLV RT, characterized by distinct structural and catalytic features compared to the wildtype MMuLV RT. Its optimal activity temperature, along with unique RNA-dependent and DNA-dependent polymerase activities, sets it apart. Notably, it exhibits a specific RNase H activity targeting RNA in RNA-DNA hybrids, which is markedly lower than that observed in Avian Myeloblastis Virus (AMV) reverse transcriptase. ™ SciPhi™ Reverse Transcriptase activity is optimal at 42°C (active up to 50°C). It is proficient in catalysing first strand cDNA synthesis, achieving a length of up to 13 kb. Additionally, the enzyme can incorporate modified nucleotides.

SciPhi™ Reverse Transcriptase: The enzyme is supplied in 0.1 M NaCl, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.1% (v/v) Triton X-100, 5 mM DTT, and 50% (v/v) glycerol.

SciPhi™ 5X RT Buffer: 250 mM Tris-HCl (pH 8.3 at 25°C), 20 mM MgCl2, 250 mM KCl, 50 mM DTT.

General Information

Definition of Activity Unit- A single unit of the enzyme incorporates 1 nmol of dTMP into a polynucleotide fraction (adsorbed on DE-81) in 10 min at 37°C.
Enzyme activity is assayed in 4 mM MgCl2, 50 mM Tris-HCl (pH 8.3), 10 mM DTT, 0.5 mM dTTP, 50 mM KCl, 0.4 MBq/mL [3H]-dTTP, 0.4 mM polyA-oligo (dT).

Inhibition and Inactivation- Inhibited by inorganic phosphate, pyrophosphate metal chelators, and polyamines. Inactivated by high temperature of 70°C for 10 min.

Source- *E.coli* cells with a cloned fragment of the *pol* gene encoding Moloney Murine Leukemia Virus reverse transcriptase.

Instruction for Use

The following steps is required to generate first-strand cDNA in two-step RT-PCR. Thaw, mix and briefly spin all the components before use and keep on ice.

1. Prepare the following mixture into sterile, DEPC treated tube on ice in the mentioned order

Template RNA	Total RNA/ Poly (A) RNA/ Specific RNA	0.1ng-5µg/ 10pg-500ng/ 0.01pg-0.5µg
Primer	Oligo(dT)$_{18}$/ Random hexamer/ gene-specific primer	0.5 µg (100 pmol)/ 0.2 µg (100 pmol)/ 15-20 pmol
DEPC-treated water		2 µL

2. ***Optional:*** In the case of RNA template with a high GC content or known secondary structures, mix gently, briefly centrifuging, and then incubating at 65°C for 5 min, chill on ice, briefly centrifuge and place on ice.

3. Add all the components in the following order:

SciPhi™ 5X RT Buffer	5 µL
RNase Inhibitor	0.5 µL (20 U)
dNTP Mix, 10 mM each	2µL (1mM final conc)
SciPhi™ Reverse Transcriptase	1 µL (200 U)
Total volume	20 µL

Mix gently followed by brief centrifugation.

4. If oligo(dT)$_{18}$ primer or gene-specific primer is used, incubate 60 min at 42°C, for random hexamer primer is used, incubate 10 min at 25°C and then 60 min at 42°C. For GC rich RNA reaction temperature can be increased to 45°C.

5. Terminate the reaction by heating at 70°C for 10 min. Avoid heat-inactivating the enzyme before analyzing long cDNA to prevent inadvertent cleavage.

Note: The reverse transcription reaction is suitable for direct use in PCR or can be stored at -20°C. Utilize 2 µL of the reaction mix for a 50 µL PCR.

Applications

- First strand cDNA synthesis for RT-PCR
- Synthesis of cDNA for cloning and expression.
- Generation of labelled cDNA probes for microarrays.
- DNA labelling.
- Analysis of RNA by primer extension.

Troubleshooting:

For troubleshooting, please email us at techteam@nextgenlife.com; info@nextgenlife.com.

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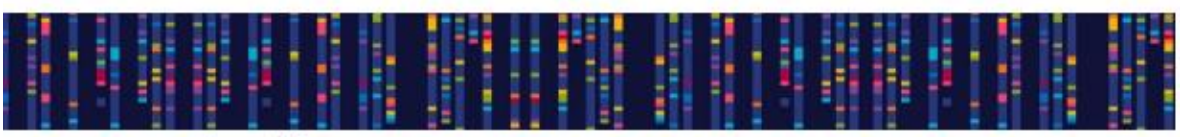

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