Lot.
Ref. SB0085

MANUAL

Expiry date: 1 year
STORE AT -20°C

REVERSE TRANSCRIPTASE (MMULV)

-Only for research use-
-To be used by a technical person-

Content: 25000 units (125µl) reverse transcriptase (200 units/µl) and 5x M-MLV reaction buffer (1ml)

Applications:
1. RT-PCR: reverse transcriptase and reaction buffer may be used in different protocols to convert RNA to cDNA. Here is an example of protocol for general reverse transcription:

   For a 50 µl reaction:

5X M-MLV Reaction Buffer	10.0 µl
dNTP mix (10mM each)	2.5 µl
Oligo (dT)12-18	1 µg
mRNA	2-5 µg
Ribonuclease Inhibitor (40 units/µl)	1.0 µl
α-32P]dNTP (S.A. > 400 Ci/mmol) if required	100 µCi
M-MLV Reverse Transcriptase (200 units/µl)	2.5 µl
Water (sterile, DEPC-Treated)	__ µl
Total Volume:	50 µl

   Incubate at 37°C for 30 min. Stop reaction by adding 2 µl of 0.5M EDTA or by heating to 75°C for 10 min. If necessary, the RNA template can be destroyed by adding 10 µl of 5M NaOH and incubating at 37°C overnight.

2. Synthesis of first strand cDNA for PCR, cloning, and hybridization probes
3. Filling-in and labeling the 3' termini of DNA with 5' protruding ends
4. Amplification of RNA
5. Primer extension assays

Description: M-MLV Reverse Transcriptase catalyzes the polymerization of DNA using template DNA, RNA or RNA:DNA hybrids. Full-length copies of large mRNAs, >10 kb, may be synthesized. M-MLV Reverse Transcriptase has a much lower RNase H activity than AMV Reverse Transcriptase resulting in high yields of full length cDNA. This makes M-MLV Reverse Transcriptase very useful in cDNA synthesis and RT-PCR.

Properties: Molecular Weight: 71 kDa (monomeric). Inhibitors: Polyamines, phosphate, pyrophosphates, and lithium chloride. Inactivation: 75°C for 10 min or by adding 2 µl of 0.5M EDTA for a 50 µl reaction.

Purity: Greater than 90% pure as determined by SDS-PAGE. Tested for contaminating endonucleases, exonucleases and ribonucleases.

Storage Buffer: 20mM Tris-HCl (pH 7.5), 0.1M NaCl, 0.1mM EDTA, 1mM DTT, 0.01% Igepal CA-630, 50% glycerol

If you should find any mistakes, please let us know. Thank you.

Suggestion: **This manual has been written specifically for beginners, hence persons with experience in PCR must use their experience to keep each step as small as possible e.g. you should calculate the amount of the needed chemicals, before starting with testing.** **Last update: 09-04-2010** **v1.1**	**Genekam Biotechnology AG** **Dammstr. 31-33** **47119 Duisburg** **Germany** **Tel. (+49) 203 / 555858-31,-32,-33** **Fax (+49) 203 / 35 82 99** **anfrage@genekam.de** **http://www.genekam.de**