Engineered M-MLV Reverse Transcriptase Basic Kit, 50 rxns (Cat No: RN012S)

Description:
Engineered M-MLV Reverse Transcriptase Basic Kit include a rationally designed M-MLV RT that exhibits increased thermal stability and reduced RNase H activity (RNase H^-) than the wild type M-MLV RT.

- Engineered M-MLV RT remains active up to 50°C increasing the length and yield of cDNA compared to WT version.
- Able to efficiently detect and produce cDNA in reactions that have less than 100 pg of RNA template.
- Able to amplify region up to 8kb.

Source:
Gene coding the respective variant is heterologously expressed in *E.coli.*

Unit Definition:
One unit is defined as the amount of enzyme needed to catalyze the incorporation of 1 nmol dTTP into acid-insoluble material in 10 min under 37°C with poly(rA) and oligo(dT).

Reagents supplied:

RN012S kit		
Cat No	**Description**	**Tubes No.**
RN001S	Engineered MMLV Reverse transcriptase, 200u/µl, 0.05ml (200 units/µl in 20 mM Tris-HCl pH 7.4, 100 mM NaCl, 0.1 mM EDTA, 50 % glycerol, 0.01% IGEPAL, 1mM DTT)	1
BR055-1	5x Reverse Transcriptase buffer, 1ml (250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM $MgCl_2$)	1
BR056-0.5	100mM DTT, 0.5ml	1
BR028-0.15	dNTPs mix 10mM each, 0.15ml	1
BR033-1	Nuclease Free Water, 1 ml	1

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Reaction guidelines and tips:

Quantities per Reaction (20µL Final Reaction Volume):

1. Preparation of Mix A	*1 ng -2 µg of total RNA** *2 µL oligod(T)$_{18}$ from 50 µM stock* §, §§ *1 µl dNTPs mix from 10 mM each stock* *X µl of Nuclease Free Water to 10 µl*
2. Incubate Mix A at 65° C for 5 min, then transfer for 1 min on ice (meanwhile prepare Mix B)	
3. Preparation of Mix B	*2,5 µl of Nuclease Free Water* *4 µl from 5x RT Buffer* *2 µl DTT from 100 mM stock* *0,5 µl of RNase inhibitor*** *1 µL of Reverse Transcriptase enzyme from stock of 200 U/µl*
4. Following Step 2, add 10 µL of Mix B to the reaction	
5. Incubate at 37° C to 55° C for **15 minutes** ***	
6. Heat inactivate the Reverse Transcriptase at 65° C for 20 min	
7. Cool down the cDNA to room temperature and use 0,1 - 2 µl in PCR reaction	

* ≤ 0,1 ng may suffice for detecting certain RNA populations that are abundant like e.g. Rubisco transcript in plants. Use 10 times less RNA quantity for coding RNAs enriched substrates.

** The final concentration of the RNase Inhibitor is 1 U/µL. Since RT enzyme and reagents have been checked for the presence of RNases and DNases, this step may be omitted if the sample is of high RNA quality.

*** Routine temp for a standard reaction is 42° C. For most RNA cases tested complete cDNA production was performed within **10-15 minutes** independently of the incubation temperature. Optimize depending on your target and provide us with your feedback to get special offers and rewards!

§ In case of random hexamers, add 2 µl from 50 µM stock per 20 µl RT reactions. Following "step2" incubate for 5 minutes at 25° C or room temperature before incubating the reaction to 42° C.

§§ In case of gene specific primer, add 2 µl from 2 µM primer stock. Incubate at 50° C for cDNA production. Optimize to your case, depending on primer's Tm. Incorporate 5-10 degrees below the Tm of your oligo. Do not incubate at temperatures higher than 55° C.

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Functional Quality Control:
Two step cDNA synthesis reaction in order to amplify an 831-bp region using 1ng Hela RNA as substrate. The resulting PCR product is visualized as a single band on a midori-stained agarose gel.

Other Quality Controls
Tested extensively for the absence of DNases and RNases and *E.coli* DNA contamination.

Shipping
Shipped on blue ice or dry ice

Storage conditions
Store at -20°C ± 5°C

Shelf life:
12 months upon production

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