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EliZyme™ Reverse Transcriptase 2.0

Intended use:

For Research Use Only. Not for use in diagnostic procedures.

Storage:

Upon arrival store components at -20 °C. Avoid prolonged exposure to light. When stored under these conditions, the kit will retain full activity until the expiration date indicated on the kit label. Avoid exposure of the stock solution to frequent temperature changes and limit handling at room temperature to the necessary minimum.

Product description

Introducing EliZyme™ Reverse Transcriptase 2.0 (RTase 2.0), a highly robust and exceptionally thermostable modified M-MLV reverse transcriptase. The enzyme can be used to synthesize cDNA at temperatures up to 65 °C, which allows reverse transcription of regions containing secondary structures with high melting temperatures. Engineered to enhance cDNA synthesis speed, yield, and representation, it excels in working with diverse RNA sample types. EliZyme™ Reverse Transcriptase 2.0 comes accompanied by an advanced 5X buffer, enriched with enhancers, dNTPs, and $MgCl_2$. This meticulously designed buffer facilitates sensitive and efficient cDNA synthesis across a wide range of RNA input amounts. With the exclusion of oligos, users are granted the freedom to tailor their own priming strategy as per their requirements. Unlike traditional obstacles, such as ribosomal and transfer RNAs, EliZyme™ Reverse Transcriptase 2.0 remains unhindered by them, thus making total RNA an ideal substrate. Whether utilizing 20 pg to 3.5 µg of total RNA or oligo(dT) purified mRNA, the RTase2.0 adapts accordingly, though the optimal template concentration is ultimately determined by the choice of oligos. To ensure the integrity of RNA during the process, the RTase 2.0 is combined with an advanced EliZyme™ RiboProtect RNase inhibitor, effectively safeguarding the RNA against degradation caused by contaminating RNase enzymes.

Content

	Ref. No.	Size	Package
EliZyme™ Reverse Transcriptase 2.0	EZ2305	1×50 µl (200 U/µl) + 1×200 µl buffer	50 rxns
	EZ2320	4×50 µl (200 U/µl) + 4×200 µl buffer	200 rxns

	Buffer/MIX	Content
EliZyme™ Reverse Transcriptase 2.0	5× buffer	15 mM $MgCl_2$, 5 mM dNTPs

Additional $MgCl_2$ or enhancers are not necessary. The buffer composition has been optimized to generate high yield, non-biased cDNA for downstream applications.

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Primers

In the following table are listed suggested primer concentrations. For non-biased, non-specific amplification is recommended to use both random hexamers and oligo $(dT)_{18}$.

Oligo type	Reaction concentration	10× Stock concentration
Specific primers	1 pM	10 pM
Random hexamers	1–5 µM	10–50 µM
Oligo $(dT)_{18}$	50–500 nM	0.5–5 µM

Reaction setup

After thawing, briefly vortex 5X EliZyme™ RT Buffer and shortly spin.

Reagent	20 µl reaction	Final conc.
5× EliZyme™ RT Buffer	4 µl	1×
Reverse transcriptase (200 U/µl)	1 µl	
Template RNA/oligo (dT) purified mRNA	20 pg – 3.5 µg*	Variable
10× Primer mix	2 µl**	1×
PCR grade water	Up to 20 µl	

* Use 20 pg to 3.5 µg total RNA or oligo(dT) purified mRNA for accurate quantification. Additional RNA is not recommended for quantification, as total reverse transcription is not guaranteed. As concentrations of target sequences will vary, users are encouraged to perform a template titration to find the optimal concentration for their application.

** Incubating template with primers prior to reverse transcription can increase the amount of cDNA, however this step is not necessary for accurate quantification. If preincubation is desired, incubate template with primers for 2 minutes at 70 °C, then rapidly cool to 4 °C, before adding to reaction.

Reverse transcription profile

Step	Temperature	Time	Cycles
Reverse transcription	50–55 °C*	10–30 min	1
Enzyme denaturation	95 °C	10 min	1

* We recommend incubating with a temperature of 50 °C for 30 minutes for most applications. Where regions of interest contain high secondary structure (>65 % GC), incubation temperatures of up to 70 °C may be used, but this will reduce the activity of the enzyme and may result in less total cDNA. The same temperature should be used when comparing samples.

Manufacturer:

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REF	Catalog number		Upper limit of temperature
LOT	Batch code		Manufacturer
	Use by (last day of month)	Σ	Contains sufficient "N" tests