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EliZyme™ OneS Kit

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. Do not store the mix once it is combined with the RTase.

Product description

The EliZyme™ OneS Kit is an all-in-one solution for cDNA synthesis and PCR that is both user-friendly and efficient. With a unique buffer system, reverse transcriptase, and hot-start polymerase, it enables specific and highly sensitive 1-step RT-PCR from any RNA sample.

The kit includes a modified thermostable reverse transcriptase that is blended with an advanced RNase inhibitor to protect RNA from degradation by any contaminating RNase. The thermostable RTase is highly active and fortified by the RNase inhibitor, and can easily process total RNA as a substrate. For PCR, the EliZyme™ OneS Kit is utilized with a hot-start technology that prevents primer-dimer formation and non-specific amplification, resulting in robust RT-PCR performance with minimal optimization. The enzyme is inactive below 65 °C and activates after heat exposure at 95 °C.

Through high-throughput screening, the buffer system has been optimized for efficient amplification from templates with varying GC content and AT content, using both fast and standard cycling conditions.

Content

	Ref. No.	Content	Size
EliZyme™ OneS Kit	EZ4505	1×1.25 ml mix + 1×0.125 ml RTase	50 rxns
	EZ4510	2×1.25 ml mix + 2×0.125 ml RTase	100 rxns
	EZ4550	2×6.25 ml mix + 1×1.25 ml RTase	500 rxns

	Buffer/MIX	Content
EliZyme™ OneS Kit	2X mix	6 mM $MgCl_2$, 2 mM dNTPs

Additional $MgCl_2$ is not necessary. The buffer composition has been optimized to maximize PCR success rates.

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Primers

Primers should have a predicted melting temperature of around 60 °C. Primers should be designed to eliminate the possibility of primer-dimer formation and non-specific amplification. The final primer concentration in the reaction should be between 0.2 µM and 0.6 µM.

PCR

The RTase also contains RNase inhibitor. For difficult templates the yield of reaction can be increased by reducing the amount of RTase added. In this case we recommend a titration (0.2X – 1X).

We recommend incubating with a temperature of 45 °C for 10 - 20 minutes for the majority of applications. Where regions of interest contain high secondary structure incubation temperatures up to 55 °C may be used. For amplicons above 1 kb the incubation time may be increased.

We recommend performing a temperature gradient to experimentally determine the optimal annealing temperature. Alternatively, we recommend a 55 °C annealing temperature then increase in 2 °C increments if non-specific products are present.

Optimal extension is achieved at 72 °C. The optimal extension time is dependent on amplicon length and complexity of template. 15 seconds per kilobase (kb) is recommended for amplification from eukaryotic DNA for amplicons between 1 kb and 3 kb.

Reaction Setup

After thawing, briefly vortex the mix and shortly spin.

Reagent	50 µl reaction	Final conc.
2X EliZyme™ OneS Kit MIX	25 µl	1×
Forward primer (10 µM)	2 µl	400 nM
Reverse primer (10 µM)	2 µl	400 nM
20x RTase	2.5 µl	1×
Template RNA	1 pg – 1 µg total RNA, > 0.01 pg mRNA	Variable
PCR grade water	Up to 50 µl	

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PCR cycling profile

Step	Temperature	Time	Cycles
Reverse transcription	45 – 55 °C*	10 min	1
Initial denaturation	95 °C	2 min	1
Denaturation	95 °C	10 s	40
Annealing	60 – 65°C	10 s	
Extension	72 °C	15 – 60 s**	

* 45 °C is recommended for most applications. 55 °C should be used only when amplicon contains regions of high secondary structure
**15 s/kb

Manufacturer:

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Catalog number

Batch code


Use by (last day of month)


Upper limit of temperature


Manufacturer

Contains sufficient "N" tests