Product Manual

AMPIGENE® 1-Step RT-PCR Kit

Catalog #: ENZ-KIT105

ENZ-KIT105-0050 for 50 rxns

ENZ-KIT105-0200 for 200 rxns

ENZ-KIT105-1000 for 1000 rxns

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Product Manual

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions.

Please contact Enzo Life Sciences Technical Support if necessary.

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DESCRIPTION

Enzo Life Sciences' AMPIGENE® 1-Step RT-PCR Kit uses the latest developments in reverse transcriptase technology and buffer chemistry for efficient cDNA synthesis and PCR in a single tube.

Our modified MMLV reverse transcriptase (RTase) is both thermostable and extremely active. The enzyme is blended with RNase inhibitor preventing degradation of RNA by contaminating RNase. The RTase is not inhibited by ribosomal and transfer RNAs, total RNA is an ideal substrate.

AMPIGENE® 1-Step RT-PCR Kit uses proprietary small molecular inhibitor technology that prevents formation of primer-dimers to improve reaction sensitivity and specificity.

High-throughput screening has resulted in a buffer system that allows efficient amplification from GC-rich and AT-rich templates, under fast and standard cycling conditions.

Component	50 reactions	200 reactions	1000 reactions
2x 1-Step Mix	1 x 1.25ml	4 x 1.25ml	20 x 1.25ml
20x RTase with RNase inhibitor	1 x 125µl	4 x 125µl	20 x 125µl

SHIPPING AND STORAGE

Protect from prolonged exposure to light.

On arrival the kit should be stored at -20°C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4°C for 1 month. The kit can go through 30 freeze/thaw cycles with no loss of activity.

IMPORTANT CONSIDERATIONS

2x 1-Step Mix: The 2x mix contains HS Taq DNA Polymerase, 6mM $MgCl_2$, 2mM dNTPs, enhancers and stabilizers. It is not recommended to add further PCR enhancers or $MgCl_2$ to the reaction. The buffer composition has been optimized to maximize PCR success rates.

20x RTase: The 20x RTase also contains RNase inhibitor. It is essential to use the correct volume per reaction. Using the incorrect volume will result in loss of sensitivity.

Template: Use 1pg to 1µg total RNA per reaction, use minimum 0.01pg mRNA per reaction.

Primers: Primers should have a predicted melting temperature of around 60°C. The final primer concentration in the reaction should be between 0.2µM and 0.6µM.

Reverse Transcription: We recommend incubating with a temperature of 45°C for 10 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 1kb the incubation time should be increased to 20 minutes.

Annealing: 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.

Extension: 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 1kb and 3kb.

REACTION SETUP

1. Before starting, briefly vortex 2x 1-Step Mix
2. Prepare a master mix based on following table; we recommend also setting up a no-RTase control:

Reagent	50µl reaction	Final concentration	Notes
2x 1-Step Mix	25µl	1x	
Forward primer (10µM)	2.0µl	400nM	See above for optimal primer design
Reverse primer (10µM)	2.0µl	400nM	
20x RTase	2.5µl	1x	Correct volume is critical, do not reduce
Template RNA	1pg to 1µg total RNA >0.01pg mRNA	variable	
PCR grade dH_2O	Up to 50µl final volume		

3. Program the instrument using following conditions:

Cycles	Temperature	Time	Notes
1	45°C to 55°C	10min	Reverse transcription, 45°C is recommended for most applications, 55°C should be used only when amplicon contains regions of high secondary structure
1	95°C	2min	Polymerase activation, 2 minutes
40	95°C 60°C to 65°C 72°C	10 seconds 10 seconds 30-60 seconds	Denaturation Anneal 15 seconds per kb

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