Product Manual

AMPIGENE® cDNA Synthesis Kit

Catalog #: ENZ-KIT106

ENZ-KIT106-0050 for 50 rxns

ENZ-KIT106-0200 for 200 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

The AMPIGENE® cDNA Synthesis Kit uses the latest developments in reverse transcriptase technology and buffer chemistry to enhance cDNA synthesis speed and yield with accurate transcript representation. The reverse transcriptase, buffer system and combination of random hexamers with anchored oligo(dT) allow for unbiased, efficient, sensitive cDNA synthesis.

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

5x buffer contains anchored oligo(dT), random hexamers, enhancers, dNTPs and $MgCl_2$. The relative concentrations of random hexamers and anchored oligo(dT) have been optimized for the generation of cDNA for use in real-time PCR experiments. The kit can be used with 4.0pg to 4.0µg total RNA.

Component	50 reactions	200 reactions
5x cDNA synthesis mix	2 x 100µl	8 x 100µl
20x RTase with RNase inhibitor	2 x 25µl	8 x 25µl

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

5x cDNA Synthesis Mix: Contains anchored oligo(dT), random hexamers, 15mM $MgCl_2$, 5mM dNTPs, enhancers and stabilizers. It is not recommended to add further enhancers or $MgCl_2$ to the reaction. The buffer composition has been optimized to generate cDNA for downstream real-time PCR analysis.

Template: For total RNA use between 4pg and 4.0µg per reaction.

Incubation temperature: We recommend incubating with a temperature of 42°C for 30 minutes for the majority of applications (<65% GC). Where regions of interest contain high secondary structure (>65% GC) incubation temperatures of up to 55°C may be used.

qPCR setup: We recommend 4.0µl of cDNA per 20µl real-time PCR reaction.

REACTION SETUP

1. Allow 5x cDNA Synthesis Mix to thaw, briefly vortex.
2. Prepare a master mix based on the following table. Insert reagents in sequence listed:

Reagent	20µl reaction	Final concentration	Notes
5x cDNA synthesis mix	4.0µl	1x	
20x RTase	1.0µl		Add before total RNA as RNAse inhibitor is blended with RTase
Total RNA (between 4pg and 4.0µg)	xµl		Variable
PCR grade dH_2O	Up to 20µl final volume		

NO RT CONTROL SETUP (OPTIONAL)

3. Prepare a master mix based on the following table. Insert reagents in sequence listed:

Reagent	20µl reaction	Final concentration	Notes
5x cDNA synthesis mix	4.0µl	1x	
Total RNA (between 4pg and 4.0µg)	xµl		Use equal amount as in step 2
PCR grade dH_2O	Up to 20µl final volume		

INCUBATION AND ENZYME DENATURATION

4. Incubate at 42°C for 30 minutes.
5. Incubate at 85°C for 10 minutes to denature RTase.

NOTES

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