AMPIGENE® Reverse Transcriptase

REF ENZ-NUC137-10001 10000 Units
REF ENZ-NUC137-40001 40000 Units

INTENDED USE:

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

SUMMARY AND EXPLANATION

AMPIGENE® Reverse Transcriptase 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 allows for efficient, non- biased and sensitive cDNA synthesis.

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

The 5x AMPIGENE® Reverse Transcriptase Buffer contains enhancers, dNTPs and $MgCl_2$. It does not contain oligos. The kit can be used with 4.0pg to 0.4µg total RNA or oligo (dT) purified mRNA. However, the optimal template concentration will ultimately be determined by what oligos are used.

ASSAY PRINCIPLE

Polymerase chain reaction (PCR) uses a polymerase enzyme, which directs the synthesis of DNA from deoxynucleotide substrates on a single-stranded DNA template, by adding nucleotides to the 3' end of a custom-designed oligonucleotide annealed to the template DNA[1].

KNOWN APPLICATION

Amplification of nucleic acid targets with PCR methods.

PRODUCTS SUPPLIED

Component	10,000 units	40,000 units
AMPIGENE® Reverse Transcriptase Buffer (5X)	1 x 200 µL	4 x 200 µL
AMPIGENE® Reverse Transcriptase (200Units/µL)	2 x 25 µL	2 x 100 µL

MATERIALS NEEDED (Not Provided)

- Target RNA
- Primers
- Thermal Cycler

STORAGE AND SHELF-LIFE

- Upon receipt, store kit at -20°C. These products are stable under these conditions up to the expiration date indicated in the vial label.
- Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months from date of receipt. 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.

PERFORMANCE CONSIDERATIONS

1. Do not use reagents past their expiration date.
2. Cross-contamination of samples could cause false results. Use care when working with more than one sample.

LIMITATIONS

- This procedure is for research use only. It is not intended for diagnostic or therapeutic use.

PRECAUTIONS

1. Refer to reagent Safety Data Sheet (SDS) from precautions.
2. Specimens, before and after fixation, and all materials exposed to them should be handled and disposed of with proper precautions.
3. Never pipette reagents by mouth and avoid contact with skin and mucous membranes with reagents and specimens. If reagents and/or specimens come into contact with sensitive areas, rinse thoroughly with water and follow your institution's safety protocols.

TECHNICAL NOTES

For technical support and troubleshooting you can submit a technical enquiry online, call us direct, or alternatively email with the following information:

- Amplicon size
- Reaction setup
- Cycling conditions
- Screen grabs of gel images

GLOBAL HEADQUARTERS

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INSTRUCTIONS FOR USE

AMPIGENE® Reverse Transcriptase Buffer. The 5X buffer contains 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 high yield, non-biased cDNA for downstream applications.

Primers: Suggested primer concentrations are in the table below. For non-biased, non-specific amplification, we recommend using both random hexamers and oligo-dT_{18}.

Oligo Type	Reaction Concentration	10X Stock Concentration
Specific Primers	1pM	10pM
Random Hexamers	2 - 5µM	20 - 50µM
Oligo-dT_{18}	1µM	10µM

Optional preincubation: Incubating primer mix with template for 5 minutes at 70°C before adding to reaction mix will increase cDNA yield. However, this step is not necessary for accurate quantification.

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.

PCR setup: We recommend 4.0µL of cDNA per 20µL real-time PCR reaction and 50µL endpoint PCR reaction

Reaction Setup

1. Allow 5X AMPIGENE® Reverse Transcriptase Buffer to thaw, briefly vortex.
2. Prepare a master mix based on the following table. Insert reagents in sequence listed:

Reagent	20 µL reaction	Final Concentration
AMPIGENE® Reverse Transcriptase Buffer (5X)	4.0 µL	1x
AMPIGENE® Reverse Transcriptase (200Units/µL)	1.0 µL	
4.0pg to 0.4µg Total RNA or oligo (dT) purified mRNA	XµL	
10x Primer Mix	2 µL	1x
PCR grade dH_2O	Up to 20 µL final volume	

No AMPIGENE® Reverse Transcriptase control setup (optional)

Reagent	20 µL reaction	Final Concentration
AMPIGENE® Reverse Transcriptase Buffer (5X)	4.0 µL	1x
4.0pg to 0.4µg Total RNA or oligo (dT) purified mRNA	X µL	
10x Primer Mix	2µL	1x
PCR grade dH_2O	Up to 20µL final volume	

Incubation and enzyme denaturation

1. Incubate at 42°C for 30 minutes.
2. Incubate at 85°C for 10 minutes to denature RTase.

REFERENCES

1. Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harb Symp Quant Biol. 1986;51 Pt 1:263-73.

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