AMPIGENE® RNase Inhibitor

REF ENZ-NUC136-2500 2500 Units

INTENDED USE:

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

SUMMARY AND EXPLANATION

AMPIGENE® RNase Inhibitor is a recombinant protein that blocks the activity of a wide range of ribonucleases to reliably protect your RNA from RNase digestion. The inhibitor is designed for use in RNA-sensitive applications such as RT-qPCR, cDNA synthesis and RNA-seq, where the presence of even small amounts of RNase can be highly detrimental to RNA quality and experimental outcome.

AMPIGENE® RNase Inhibitor has a molecular weight of 50 kDa and is purified from High Five insect cells expressing a modified human placental gene. The inhibitor binds noncovalently to RNases at a 1:1 ratio, and has a Ki value of approximately 10–14 M when binding to RNase A[1]. Moreover, the very rapid kinetics of association to RNases guarantees immediate protection of your RNA.

Some cysteine residues present in human placental protein have been implicated in the oxidation sensitivity of the protein[2]. **AMPIGENE® RNase inhibitor** does not contain these residues, resulting in a molecule more resistant to oxidative stress.

The high thermostability of ensures activity up to 65 °C for 30 minutes. The inhibitor can block the activity of a wide range of ribonucleases, including eukaryotic RNases of the neutral type (e.g. RNases A, B and C). It does not inhibit RNases T1, T2, U1, 2, CL3, RNase I and H. The inhibitor is free from ribonucleases and phosphatases, and is inactivated by heating at 75 °C for 15 minutes.

ASSAY PRINCIPLE

AMPIGENE® RNase inhibitor can be used in the polymerase chain reaction (PCR). 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[3].

KNOWN APPLICATION

Amplification of nucleic acid targets with PCR methods.

PRODUCTS SUPPLIED

Component	2500 units
AMPIGENE® RNase Inhibitor (40 U/µL)	1 x 62.5 µL

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

We recommend adding 40 units of **AMPIGENE® RNase inhibitor** to a 20 µL reaction (1 µL per reaction to work with a final concentration of 2 U/µL). Titration may be required in case of templates derived from RNase-rich sources.

For RT-qPCR reactions, our kits already contain enough RNase inhibitor to protect the RNA template in most of the cases. However, for templates derived from RNase-rich sources we recommend supplementing the reaction with additional 0.4 U/µL RNase inhibitor (i.e. adding extra 0.2 µL RNase inhibitor to a 20 µL reaction).

AMPIGENE® RNase inhibitor can be used to prevent RNA degradation after extraction (to prolong RNA viability during storage). In this case, we recommend using AMPIGENE® RNase inhibitor as a 100X solution (i.e., the concentration of RNase inhibitor in the storage buffer should be 0.4 U/µL). Once again, higher amounts may be required in case of templates derived from RNase-rich sources.

AMPIGENE® RNase inhibitor can also be used to block RNA degradation during RNA extraction in all those methods which do not include a protein denaturation step, given the proteic nature of the inhibitor itself. Also in these cases, the final concentration should be ≥0.4 U/µL.

REFERENCES

1. Lee FS, Shapiro R, Vallee BL. Tight-binding inhibition of angiogenin and ribonuclease A by placental ribonucleasenhibitor. Biochemistry. 1989; 28:225–230.
2. Kim BM, Schultz LW, Raines RT. Variants of ribonuclease inhibitor that resist oxidation. Protein Science. 1999; 8(2):430-434.
3. 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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