RT35L

RIBOPROTECT Hu RNase Inhibitor / Lyo-ready









The *RIBOPROTECT Hu* RNase Inhibitor Lyo-ready is a 50 kDa recombinant human placental protein expressed in *Escherichia coli* in a special formulation (without glycerol*). It inhibits ribonuclease (RNase) activity of common eukaryotic enzymes such as RNase A, RNase B, RNase C by non-covalent binding in a 1:1 ratio. *RIBOPROTECT Hu* is intended for use in applications where the presence of RNases may cause a hazard to RNA quality and experiment results, e.g. in RNA isolation, cDNA synthesis, RT-PCR, *in vitro* transcription and translation RT-qPCR, RT-LAMP or RNase-free monoclonal antibody preparation. *RIBOPROTECT Hu* shows no activity towards RNase 1, RNase T1, RNase T2, S1 nuclease and RNase H. It is compatible with DNA Polymerases and AMV or M-MuLV Reverse Transcriptases. Formulation of *RIBOPROTECT Hu* Lyo-ready enables its usage directly in the lyophilization process.

* Trace amounts of glycerol may be present



Features and advantages

- → Glycerol-free formulation^{*} ready for lyophilization
- → Full stability at 37°C for at least 4 weeks and at 50°C for at least 6 hours.
- → Completely inhibits RNase A, B and C activity
- → No influence on the polymerase or reverse transcriptase activity
- → Free of DNase and RNase activity
- → Active in diverse reaction conditions and in various buffers
- → Active over a broad pH (pH 5.5 9.0) and DTT ranges

Applications

- → cDNA synthesis, RT-PCR, RT-qPCR, RT-LAMP
- → developing lyophilized diagnostic kits for optimal RNA protection
- → RNA isolation and purification
- → *in vitro* transcription and translation
- → RNase-free monoclonal antibody preparation



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Usage

- → The optimal final concentration of the *RIBOPROTECT Hu* in a reaction depends on the level of RNase contamination, the incubation time and the compounds present in the reaction mixture. It falls within a range of 1–2 U/µl.
- → For a standard reverse transcription reaction, use 40 U of the *RIBOPROTECT Hu* for the final sample volume of 20 µl.
- → For an optimal *RIBOPROTECT Hu* activity, the final DTT (or other reducing agent) concentration of 0.5-1 mM is essential.
- → During assembly of a reaction, *RIBOPROTECT Hu* should be added before other components that are possible sources of RNase contamination.
- → Using *RIBOPROTECT Hu* does not exclude RNase H treatment after amplification of the first strand cDNA.



Quality control

The absence of Endonuclease, Exonuclease, RNase and latent RNase activities has been confirmed using the relevant procedures. The purity is >90% as judged by SDS-polyacrylamide gels.

Unit definition

One unit is defined as the amount of enzyme required to inhibit the activity of 5 ng RNase A by 50%.



Additional information

- → 0.5-1 mM DTT (or other reducing agent) presence is essential for optimal activity of the *RIBOPROTECT Hu* RNase Inhibitor.
- → The storage buffer contains 8 mM reducing agent, however, if the ratio of the RNase inhibitor to the final sample volume is less than 1:8, then the addition of DTT (or other reducing agent) to a final concentration of 0.5-1 mM is recommended.

Storage buffer

20 mM HEPES-KOH (pH 7.6); 50 mM KCl; 8 mM reducing agent



Troubleshooting

Problem	Possible cause	Solution	
No RNase Inhibitor activity	RIBO PROTECT Hu shows no activity towards the RNases present in the sample	Maintain aseptic working conditions. Use disposable gloves, changing them as frequently as required. Use RNase-free consumables. Only work in an area assigned for working with RNA and with equipment designated for that purpose. Use a different RNase inhibitor.	
	DTT (or other reducing agent) concentration is too low	Add the required quantity of DTT (or other reducing agent) to a final concentration of 0.5-1 mM.	
	No activity owing to denaturating conditions	Avoid conditions which compromise the <i>RIBOPROTECT Hu</i> activity. It is inhibited by denaturating agents, such as SDS, urea and oxidising substances.	



Component	RT35L-010 10 000 U	RT35L-B 1000 U
<i>RIBOPROTECT Hu RNase Inhibitor Lyo-ready (40 U/ul)</i>	250 µl	bulk volume upon request

Storage & shipping

Storage conditions

Store at $2-8^{\circ}$ C for up to several weeks, for a long term storage -80° C is recommended, up to 3 freeze/thaw cycles is acceptable.

Shipping conditions

Shipping on blue ice.

(i) For research use only

Expiry

Information on the label.



Made in Poland