

Ribonuclease Inhibitor (Porcine liver)

Code No. 2311A **Size:** **5,000 U**
Conc.: **40 U/μl**

Description :

RNase Inhibitor is purified from porcine liver by affinity chromatography on an immobilized RNase A column. The character of this enzyme is similar to the one from human placenta.^{1, 2)} It forms a 1 : 1 complex with RNase A, and inhibits RNase activity noncompetitively ($K_i = 4 \times 10^{-10}$ M).³⁾ This reaction is reversible, and the ribonuclease activity is recovered by dissociating the complex with urea or sulfhydryl reagent. In this case, Inhibitor is inactivated irreversibly. It can be added directly to reaction mixtures containing RNA. Moreover, because it is a protein, it differs from other competitive inhibitors (nucleotides and inorganic phosphatases) in that it can easily be removed from the reaction system by phenol extraction. However, it does not inhibit the RNase H activity of Reverse Transcriptase. This product can be used in the same applications as the one from human placenta. Therefore, it is useful in applications where human DNA contamination is a concern.

Storage Buffer :

20 mM	HEPES-KOH (pH 7.5)
50 mM	KCl
5 mM	DTT
50%	Glycerol

Storage: -20°C

Source: Porcine liver

Unit definition :

One unit is the amount of the inhibitor required to inhibit by 50% the activity of 5 ng of RNase A (This inhibitor activity is determined by its ability to inhibit the hydrolysis of cyclic 2', 3'-CMP by RNase A).⁴⁾

Quality Control Data :

Please see the Certificate of Analysis (CoA) for each lot. You can download the CoA on Takara Bio website.

Note :

It inhibits in a wide pH range, but most strongly at pH 7 - 8.
It requires DTT of at least 1 mM to be active.

Applications :

1. cDNA synthesis (Ribonuclease Inhibitor, 0.5 U/μl reaction)⁵⁾
2. *In vitro* translation (Ribonuclease Inhibitor, 1 U/μl reaction)⁶⁾
3. *In vitro* transcription with cell-free extract (Ribonuclease Inhibitor, 20 U/μl reaction)⁷⁾
4. *In vitro* transcription with SP6 or T7 RNA polymerase (Ribonuclease Inhibitor, 1 U/μl reaction)⁷⁾
5. Polysome isolation (Ribonuclease Inhibitor, 1,000 U/ml reaction)⁶⁾

References :

- 1) Burton L E and Fucci N P. *Int J Pept Protein Res.* (1982) **19**: 372-379.
- 2) Blackburn P, Wilson G, and Moore S. *J Biol Chem.* (1977) **252**: 5904-5910.
- 3) Turner P M, Lerea K M, and Kull F J. *Biochem Biophys Res Comm.* (1983) **114**: 1154-1160.
- 4) Blackburn, P. *J Biol Chem.* (1979) **254**: 12484-12487.
- 5) de Martynoff G, Pays E, and Vassart G. *Biochem Biophys Res Comm.* (1980) **93**: 645-653.
- 6) Scheele G and Blackburn P. *Proc Natl Acad Sci USA.* (1979) **76**: 4898-4902.
- 7) Eichler D C, Tatar T F, and Lasater L S. *Biochem Biophys Res Comm.* (1981) **101**: 396-403.

Note

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Ribonuclease Inhibitor (Porcine liver)

Code No. 2311A 容量： 5,000 U
 濃度： 40 U/ μ l

●製品説明

RNase Inhibitor は、ブタ肝臓より固定化 RNase A カラムを利用してアフィニティー精製したものであり、Human placenta 由来の RNase inhibitor と非常によく似た性質を持つ。^{1,2)} RNase A と 1 : 1 の複合体を形成し、ribonuclease 作用に対し高い非拮抗阻害 ($K_i = 4 \times 10^{-10}$ M) を示す。³⁾ しかし、この反応は可逆的であり、尿素あるいは sulfhydryl 試薬で複合体を解離させることにより ribonuclease 作用は復活し、inhibitor は不可逆的に失活する。また従来の拮抗性阻害剤 (ヌクレオチド類、無機リン酸類) とは異なり、タンパク性であるので、反応系からフェノール処理により容易に除くことが出来る。なお、RNase H 活性は阻害しない。本製品は、Human placenta 由来の RNase inhibitor と同様に使用できる。

●形状

20 mM HEPES-KOH (pH7.5)
50 mM KCl
5 mM DTT
50% Glycerol

●保存 - 20°C

●起源 Porcine liver

●活性の定義

5 ng の RNase A の活性を 50% 阻害する活性を 1 U とする。
(Cyclic 2',3'-CMP から RNase A により生成する 3'-CMP を定量)⁴⁾

●品質管理データ

性能試験結果については、各ロットの Certificate of Analysis (CoA) をご覧ください。CoA はタカラバイオウェブサイトからダウンロードできます。

●使用上の注意

阻害活性は広い pH 域で見られ、pH7 ~ 8 で最大となる。活性発現のためには少なくとも 1 mM の DTT を必要とする。

●用途

1. cDNA 合成反応 (Ribonuclease Inhibitor 0.5 U/ μ l reaction)⁵⁾
2. *in vitro* translation (Ribonuclease Inhibitor 1 U/ μ l reaction)⁶⁾
3. *in vitro* transcription with cell-free extract (Ribonuclease Inhibitor 20 U/ μ l reaction)⁷⁾
4. *in vitro* transcription with SP6 or T7 RNA polymerase (Ribonuclease Inhibitor 1 U/ μ l reaction)⁷⁾
5. Polysome isolation (Ribonuclease Inhibitor 1,000 U/ml reaction)⁶⁾

●参考文献

- 1) Burton L E and Fucci N P. *Int J Pept Protein Res.* (1982) **19**: 372-379.
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- 4) Blackburn, P. *J Biol Chem.* (1979) **254**: 12484-12487.
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- 6) Scheele G and Blackburn P. *Proc Natl Acad Sci USA.* (1979) **76**: 4898-4902.
- 7) Eichler D C, Tatar T F, and Lasater L S. *Biochem Biophys Res Comm.* (1981) **101**: 396-403.

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