

Product Data Sheet

Product Name: RNase A, Bovine pancreas

Cat. No.: GE10005

Chemical Properties

Cas. No. 9001-99-4

Formula M.Wt ~13700

Solubility Water : >100 mg/mL Storage Store at -20°C; Need to be dry

General tips For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.

Structure



Protocol

Application Method of RNase A, Bovine Pancreas in Ribonuclease Protection Assay (RPA) [1]

This experimental protocol is compiled based on research content and is for reference only; please adjust according to actual needs.

(1) Reagent preparation: Dissolve RNase A, Bovine pancreas in an appropriate buffer (e.g., 10mM Tris-HCl, pH7.5, 300mM NaCl, 5mM EDTA) to prepare a stock solution (e.g., 10mg/mL); mix with RNase T1 to prepare the RNase digestion mixture (final concentrations typically RNase A, Bovine pancreas 40µg/mL, RNase T1 2µg/mL); prepare radiolabeled (e.g., ³²P-UTP) antisense RNA probes

Caution: Product has not been fully validated for medical applications. For research use only.

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(length 150-500nt), purify and set aside; synthesize unlabeled sense RNA (cRNA) at known concentrations as absolute quantification standards.

(2) Probe and sample preparation: Clone the cDNA fragment corresponding to the target mRNA into a plasmid containing a phage RNA polymerase promoter (SP6, T3 or T7); linearize the plasmid with restriction enzyme digestion, then perform in vitro transcription to obtain antisense RNA probes (specific activity approximately 10^9 cpm/ μ g); total RNA samples (typically 1-10 μ g) are used directly for liquid hybridization without electrophoretic separation.

(3) Hybridization: Mix an appropriate amount of radiolabeled probe (approximately 5×10^5 cpm) with the RNA sample (or serially diluted cRNA standards) in hybridization buffer; incubate at a suitable temperature (usually 42-45°C) overnight (12-16h) to allow sufficient formation of RNA-RNA hybrids between the probe and target mRNA.

(4) RNase digestion: After hybridization, add the RNase digestion mixture (containing RNase A, Bovine pancreas and RNase T1); incubate at 30-37°C for 30-60min to specifically degrade unhybridized single-stranded RNA probe and sample RNA; then terminate digestion by adding proteinase K and SDS; extract with phenol/chloroform and precipitate with ethanol to recover RNase-resistant hybrids.

(5) Product analysis: Dissolve the precipitated hybrids in loading buffer, separate by denaturing polyacrylamide gel electrophoresis (PAGE), dry the gel, and detect protected fragment signals by autoradiography or phosphor imaging; alternatively, directly precipitate RNase-digested hybrids onto filters for scintillation counting quantification. Compare with the cRNA standard curve to determine the absolute content of target mRNA in the sample.

Precautions:

(1) RPA sensitivity is at least 10-fold higher than Northern blot, allowing detection of low-abundance mRNA (as low as 5pg).

(2) The optimal probe length is 150-500nt; too short results in weak signals, while too long tends to produce smeared bands due to radioactive decay-induced fragmentation.

(3) For your safety and health, wear laboratory coat and disposable gloves during operation; radioactive isotopes must be handled in accordance with regulatory protection measures.

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References:

[1] Reue K. mRNA quantitation techniques: considerations for experimental design and application. J Nutr. 1998 Nov;128(11):2038-44.

Background

RNase A, Bovine pancreas is an endoribonuclease derived from bovine pancreas with high thermal stability. RNase A, Bovine pancreas specifically cleaves the phosphodiester bond at the 3' end of pyrimidine nucleotides (C, U) in single-stranded RNA to degrade RNA, while remaining resistant to high temperature and reducing conditions to retain enzyme activity. RNase A, Bovine pancreas can be used in studies related to plasmid DNA purification, RNA removal, and molecular biology^[1-4].

References:

- [1] Seo Y, Jun HR, Lee J, et al. In-Cell RNA Hydrolysis Assay: A Method for the Determination of the RNase Activity of Potential RNases. Mol Biotechnol. 2015 Jun;57(6):506-12.
- [2] Nguyen TT, Smith JB. Ribonuclease protection assay streamlined for high throughput with RNA in formamide and single-step rnase inactivation. Biotechniques. 2002 Mar;32(3):502, 504, 506.
- [3] Dang W, Luo R, Fan J, et al. RNase A activity analysis and imaging using label-free DNA-templated silver nanoclusters. Talanta. 2020 Mar 1;209:120512.
- [4] Nogués MV, Vilanova M, Cuchillo CM. Bovine pancreatic ribonuclease A as a model of an enzyme with multiple substrate binding sites. Biochim Biophys Acta. 1995 Nov 15;1253(1):16-24.

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