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RNase, DNase-free, High Concentration from bovine pancreas

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Cat. No. 11 579 681 001 1 mg 10 mg/ml

Store the product at -15 to -25°C.

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1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	RNase, DNase-free, high concentration	 Solution in 10 mM Tris-HCl, 5 mM CaCl₂, 50% glycerol, pH 7.0. Heterogeneous mixture of ribonucleases, DNase-free. 	1 vial, 1 mg (100 μl)

1.2. Storage and Stability

Storage Conditions (Product)

When stored at -15 to -25°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	RNase, DNase-free, high concentration	Store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

For isolation of genomic DNA

- SDS*
- Tris-HCI*
- EDTA
- Proteinase K*
- NaCl
- Phenol/chloroform
- Ethanol

1.4. Application

Use RNase, DNase-free in DNA isolation procedures.

- No boiling to remove DNases or other pretreatment is necessary.
- This high-concentration enzyme is particularly useful in the isolation of genomic DNA.

2. How to Use this Product

2.1. Protocols

Isolation of genomic DNA

High-concentration RNase, DNase-free, can be used in the isolation of genomic DNA. Use the following protocol as a starting point. Optimize this protocol, for example, by changing the concentration of RNase, DNase-free or incubation times for different cell types.

- 1 Trypsinize, harvest, and resuspend cells at a concentration of 1 to 2 × 10⁷/ml of 10 mM Tris-HCl*, pH 8.0, 10 mM EDTA.
- 2 Add sodium dodecyl sulfate (SDS)* to a final concentration of 0.5% and Proteinase K* to a final concentration of 200 μg/ml.
- 3 Vortex and incubate at +55°C for at least 2 hours.
- Add NaCl to a final concentration of 0.2 M.
- 5 Extract twice with equilibrated phenol/chloroform (mixed 1:1) and once with chloroform.
- 6 Place the tube, with its top off, in a water bath at +55°C for 1 hour to evaporate the chloroform.
- Add RNase, DNase-free, to a final concentration of 50 µg/ml and incubate at +37°C for 1 hour.
- 8 Extract once with equilibrated phenol/chloroform (mixed 1:1), and once with chloroform.
- 9 Precipitate the DNA with 1.5 volumes of ethanol.
- 10 Centrifuge the tube at 10,000 x g to pellet the DNA.
- Resuspend the pellet in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.

Isolation of plasmid DNA

High-concentration RNase, DNase-free, can also be used in isolation of plasmid DNA. The optimal working concentration for RNase, DNase-free is 2 to $5 \mu g/ml$.

2.2. Parameters

Specific Activity

 \geq 30 units/mg dry powder with one Kunitz unit (+25°C, RNA as substrate) being the amount of enzyme that causes a decrease in absorbance of A₀ to A₁ within 1 minute under assay conditions. A₀ to A₁ corresponds to the total conversion. A₁ being the final absorbance.

Working Concentration

Isolation of genomic DNA: 50 μ g/ml Isolation of plasmid DNA: 2 to 5 μ g/ml

3. Additional Information on this Product

3.1. Test Principle

Preparation

RNase, DNase-free, is isolated from bovine pancreas and purified by column chromatography.

Absence of DNase activity

RNase, DNase-free (1mg/ml) is incubated with 1 μ g of pUC19 DNA at +37°C for 6 hours in 20 μ l of restriction enzyme buffer. The DNA is analyzed by agarose-gel electrophoresis and ethidium-bromide staining; no increase in the amount of linear or relaxed circular DNA is seen.

3.2. Quality Control

For lot-specific certificates of analysis, see section, Contact and Support.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols					
i Information Note: Additional information about the current topic or procedure.					
⚠ Important Note: Information critical to the success of the current procedure or use of the product.					
1 2 3 etc.	Stages in a process that usually occur in the order listed.				
1 2 3 etc.	Steps in a procedure that must be performed in the order listed.				
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.				

4.2. Changes to previous version

Layout changes. Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.	
Reagents, kits			
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001	
Tris hydrochloride	500 g	10 812 846 001	
Proteinase K, recombinant, PCR Grade	1.25 ml, > 50 U/ml	03 115 887 001	
	5 ml, > 50 U/ml	03 115 828 001	
	25 ml, > 50 U/ml	03 115 844 001	

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to: **List of biochemical reagent products**.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

