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

RNase, DNase-free
from bovine pancreas

Version: 08
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Cat. No. 11 119 915 001	500 µg 1 ml

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	RNase, DNase-free	▪ Solution in 10 mM Tris-HCl, 5 mM $CaCl_2$, 50% glycerol; pH 7.0. ▪ Heterogeneous mixture of ribonucleases prepared free of deoxyribonuclease activity. ▪ Protein concentration, 500 µg/ml.	1 vial, 1 ml

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	Store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

For preparation of working solutions

See section, **Working Solution** *for information on preparing solutions.*

- Autoclaved, double-distilled water
- Glucose
- EDTA
- Tris-HCl*
- NaOH
- SDS
- Glacial acetic acid
- Potassium acetate

For small-scale plasmid preparation

See section, **Working Solution** *for information on preparing solutions.*

- 15 ml plastic centrifuge tube
- Autoclaved, microcentrifuge tube
- Toothpick
- Culture medium, such as LB broth
- Antibiotic, such as Ampicillin*
- Shaker
- 70% ethanol
- Absolute ethanol
- Phenol/chloroform
- Chloroform/isoamyl alcohol
- NaCl or sodium acetate
- Autoclaved, double-distilled water or buffer

For large-scale plasmid preparation

See section, **Working Solution** *for information on preparing solutions.*

- 15 ml plastic centrifuge tube
- Autoclaved, microcentrifuge tube
- Toothpick
- Culture medium, such as LB broth
- Antibiotic, such as Ampicillin*
- Shaker
- 70% ethanol
- Absolute ethanol
- Phenol/chloroform
- Chloroform/isoamyl alcohol
- NaCl or sodium acetate
- Autoclaved, double-distilled water
- Lysozyme

1.4. Application

RNase, DNase-free is particularly well suited for use in DNA isolation procedures.

2. How to Use this Product

2.1. Before you Begin

Working Solution

Solution	Preparation/Composition
Solution 1	50 mM glucose, 10 mM EDTA, 25 mM Tris-HCl*, pH 8.0.
Solution 2 (0.2 M NaOH, 1% (w/v) SDS*)	Mix 3.5 ml autoclaved, double-distilled water, 1 ml of 1 M NaOH, and 0.5 ml of 10% SDS (w/v). ⚠ ***Keep solution at +15 to +25°C; prepare just before use.***
Solution 3 (3 M potassium acetate, pH 4.8)	Mix 11.5 ml of glacial acetic acid, 28.5 ml autoclaved, double-distilled water, and 60 ml of 5 M potassium acetate.

2.2. Protocols

Small-scale plasmid preparation (miniprep)

⚠ ***Note: RNase, DNase-free, does not need to be boiled to remove DNase; it can be used directly from the storage vial. The protocol described here yields 2 to 3 µg plasmid DNA.***

See section, **Working Solution** *for additional information on preparing solutions.*

1. To a 15 ml plastic centrifuge tube, add 5 ml of culture medium (LB broth plus an antibiotic, such as 50 µg ampicillin/ml), to maintain the plasmid.
   – Use a toothpick to remove a colony of plasmid-bearing *E. coli* from a Petri dish and resuspend the colony in the 5 ml culture medium.
   – Place the tube on a shaker and grow the cells at +37°C overnight.

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2. Pour 1.5 ml of the overnight culture into a microcentrifuge tube and centrifuge for 1 minute.
   – Discard as much of the supernatant as possible.

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3. Resuspend the cell pellet in 100 µl of Solution 1 and incubate at +15 to +25°C for 5 minutes.

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4. To the tube, add 200 µl of freshly prepared Solution 2.
   – Mix the contents of the tube gently by inversion.
   – Incubate on ice for 5 minutes.

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5. To the tube, add 150 µl of Solution 3; mix gently.
   – Incubate on ice for 5 minutes.
   A white precipitate will form.

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6. Centrifuge the suspension in a microcentrifuge at 12,000 × *g* for 5 minutes at +2 to +8°C.
   – Transfer the plasmid-containing supernatant to an autoclaved microcentrifuge tube.

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7. Add 2.5 volumes, approximately 450 µl, of cold absolute ethanol to the supernatant and incubate at −60°C or below for 30 minutes to precipitate the DNA.
   – Centrifuge the suspension at 12,000 × *g* for 10 minutes at +2 to +8°C.
   – Discard the supernatant and wash the pellet with cold 70% ethanol.
   – Dry the pellet under vacuum.

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8. Resuspend the pellet in 50 µl of autoclaved, double-distilled water.
   – Add 0.5 µl of RNase, DNase-free.
   – Incubate at +37°C for 30 minutes.

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9. Extract the sample twice with phenol/chloroform, then twice with chloroform/isoamyl alcohol.
   – For each extraction, mix the DNA sample with an equal volume of phenol/chloroform or chloroform/isoamyl alcohol until an emulsion forms.
   – Centrifuge the tube at 12,000 × *g* for approximately 15 seconds at +15 to +25°C.
   – The upper aqueous phase contains the DNA and should be transferred to a fresh autoclaved microcentrifuge tube after each extraction.

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10. Add sodium chloride (final concentration, 0.1 M) or sodium acetate (final concentration, 0.25 M) to the sample.
    – Precipitate the DNA by adding 2.5 volumes of cold absolute ethanol.
    – Incubate for 30 minutes at −60°C or below, then centrifuge at 12,000 × *g* for 10 minutes at +2 to +8°C.

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11. Discard the supernatant, dry the DNA pellet under vacuum, and resuspend the pellet in a volume of approximately 20 µl autoclaved double-distilled water or buffer.
    – DNA from this miniprep is ready for sequencing or digestion with restriction endonucleases.
    This procedure can be scaled up. When processing cells from >50 ml of culture medium, add 5 mg lysozyme/ml at Step 3.*

Large-scale plasmid preparation (maxiprep)

See section, **Working Solution** *for additional information on preparing solutions.*
To obtain plasmid from the cells in a 100 ml bacterial culture, follow the steps below.

1. To a 15 ml plastic centrifuge tube, add 5 ml of culture medium (LB broth plus an antibiotic, such as 50 µg ampicillin*/ml), to maintain the plasmid.
   – Use a toothpick to remove a colony of plasmid-bearing *E. coli* from a Petri dish and resuspend the colony in the 5 ml culture medium.
   – Place the tube on a shaker and grow the cells at +37°C overnight.

2. Pellet the cells from the entire 100 ml culture by centrifugation.
   – Discard as much of the supernatant as possible.

3. Resuspend cell pellet in 2 ml of Solution 1 containing 5 mg/ml lysozyme*.
   – Transfer cell suspension to a 15 ml centrifuge tube.

4. Add 4 ml of Solution 2.
   – Mix the contents of the tube gently by inversion.
   – Incubate on ice for 10 minutes.

5. Add 3 ml of Solution 3.
   – Incubate on ice for 10 minutes.
   – A white precipitate will form.

6. Centrifuge the suspension in a microcentrifuge at 12,000 × *g* for 5 minutes at +2 to +8°C.
   – Transfer the plasmid-containing supernatant to an autoclaved microcentrifuge tube.

7. Add 2.5 volumes, approximately 450 µl of cold absolute ethanol to the supernatant and incubate at −60°C or below for 30 minutes to precipitate the DNA.
   – Centrifuge the suspension at 12,000 × *g* for 10 minutes at +2 to +8°C.
   – Discard the supernatant and wash the pellet with cold 70% ethanol.
   – Dry the pellet under vacuum.

8. Resuspend the pellet in 2 ml autoclaved, double-distilled water and add 8 µl of RNase, DNase-free.
   – Incubate at +37°C for 30 minutes.

9. Extract the sample twice with phenol/chloroform, then twice with chloroform/isoamyl alcohol.
   – For each extraction, mix the DNA sample with an equal volume of phenol/chloroform or chloroform/isoamyl alcohol until an emulsion forms.
   – Centrifuge the tube at 12,000 × *g* for approximately 15 seconds at +15 to +25°C.
   – The upper aqueous phase contains the DNA and should be transferred to a fresh autoclaved microcentrifuge tube after each extraction.

10. Add sodium chloride (final concentration, 0.1 M) or sodium acetate (final concentration, 0.25 M) to the sample.
    – Precipitate the DNA by adding 2.5 volumes of cold absolute ethanol.
    – Incubate for 30 minutes at −60°C or below, then centrifuge at 12,000 × *g* for 10 minutes at +2 to +8°C.

11. Discard the supernatant, dry the DNA pellet under vacuum, and resuspend the pellet in a volume of approximately 20 µl autoclaved double-distilled water or buffer.
    – DNA from this maxiprep is ready for sequencing or digestion with restriction endonucleases.

Genomic DNA Isolation

For every 5 × 10^7 eukaryotic cells, use 8 µl of RNase, DNase-free.

2.3. Parameters

Working Concentration

The optimal working concentration for RNase, DNase free, is 2 to 5 µg/ml. The reaction volume will vary for different applications.
Some suggested guidelines are given below:

Application	Amount of RNase, DNase-free [µl]	Reaction Volume
Small-scale isolation of plasmid DNA (miniprep) from 1.5 ml bacterial culture.	0.5	50 µl
To isolate plasmid DNA (maxiprep) from a 100 ml bacterial culture.	8	2 ml
To isolate genomic DNA from 5 × 10^7 cultured mammalian cells.	8	2 ml

See section, **Protocols***, for additional information.*

3. Additional Information on this Product

3.1. Test Principle

Alkaline lysis method (miniprep)

The alkaline method is a fast, easy way to purify small amounts of plasmid DNA.

① Plasmid-containing cells are lysed with SDS at high pH.
– When the pH is adjusted to neutrality, the plasmid DNA renatures, but the chromosomal DNA does not.

② When potassium acetate is added to the preparation, the denatured chromosomal DNA complexes with protein and SDS.

③ Centrifugation removes the insoluble protein-DNA-SDS complex, but leaves the plasmid DNA, along with RNA, in solution.

④ RNase, DNase-free, hydrolyzes the RNA without damaging the plasmid DNA.

Preparation

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

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	
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.
❶ ❷ ❸ 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		
Tris hydrochloride	500 g	10 812 846 001

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.

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