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RNase One Ribonuclease digestion

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ABSTRACT

Modified from: http://www.promega.com/paguide/chap6.htm#RNaseAmount

This protocol was used to remove RNA from DNA preparations.

The RNases commonly used in an RPA are RNase I , RNase T1 or a combination of RNase T1 and RNase A. RNase I cleaves after all four ribonucleotides, RNase T1 cleaves after G residues and RNase A cleaves after A and U residues. For most efficient cleavage of the single-stranded regions immediately adjacent to the double-stranded RNA, we recommend RNase I (RNase ONE™ Ribonuclease, Cat.# M4261).

Use 1–10u RNase ONE™ Ribonuclease per 10µg of total RNA or 0.1–1u of RNase ONE™ Ribonuclease per 1µg of poly(A)+ RNA.

PROTOCOL CITATION

Roey Angel, Eva Petrova 2020. RNase One Ribonuclease digestion. **protocols.io** https://protocols.io/view/rnase-one-ribonuclease-digestion-qdzds76

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GUIDELINES

Annealing and Digestion Temperatures

RNase ONE™ Ribonuclease digestion works most efficiently at 20–37°C (Brewer *et al.* 1992).

MATERIALS

NAME	CATALOG #	VENDOR
Roti®-Phenol/Chloroform/Isoamyl alcohol ()	A156.3	Carl Roth
RNase ONE™ Ribonuclease	M4261	Promega
Proteinase K Solution (20 mg/mL)	AM2548	Thermo Fisher Scientific
Gel Loading Dye Purple (6X) - 4.0 ml	B7024S	New England Biolabs
Yeast tRNA (10 mg/mL)	AM7119	Thermo Fisher Scientific

STEPS MATERIALS

NAME	CATALOG #	VENDOR
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Proteinase K Solution (20 mg/mL)	AM2548	Thermo Fisher Scientific
Roti®-Phenol/Chloroform/Isoamyl alcohol ()	A156.3	Carl Roth
Yeast tRNA (10 mg/mL)	AM7119	Thermo Fisher Scientific

BEFORE STARTING

Materials Required:

- purified total RNA or poly(A)+ RNA from the tissue or cells of interest
- 3.0M ammonium acetate (pH 5.2)
- ice-cold 100% ethanol
- ice-cold 70% ethanol
- 20% w/v SDS
- RPA loading dye

1 Incubate 5–10µg of total NA 85°C for 5 minutes to denature the NAs.

5 µg total NA

85 °C

00:05:00

2 Add 300µl of RNase digestion buffer and the appropriate amount of RNase ONE™ Ribonuclease (1-10u per 10µg of total RNA). Incubate the samples for 30–60 minutes at 20–37°C.

300 µl RNase digestion buffer

RNase ONE™ Ribonuclease
by Promega
Catalog #: M4261

00:30:00

20 °C

3 Stop the reaction as follows: Add 10µl of 20% w/v SDS and 2.5µl of 20mg/ml proteinase K. Incubate for 15 minutes at 37°C.

10 µl 20% w/v SDS

37 °C

00:15:00

2.5 µl 20mg/ml proteinase K

Proteinase K Solution (20 mg/mL)
by Thermo Fisher Scientific
Catalog #: AM2548

4 Extract once with phenol:chloroform:isoamyl alcohol, and remove the aqueous phase to a clean microcentrifuge tube containing 1µl of 10mg/ml carrier tRNA .

Roti®-Phenol/Chloroform/Isoamyl alcohol ()
by Carl Roth
Catalog #: A156.3

1 µl 10mg/ml carrier tRNA

A yeast tRNA is an effective coprecipitant to aid in recovery of small amounts of nucleic acids.

Yeast tRNA (10 mg/mL)
by Thermo Fisher Scientific
Catalog #: AM7119

5 Add 825µl of ice-cold 100% ethanol, and chill at –20°C for 30 minutes, then centrifuge at maximum speed in a microcentrifuge for 15 minutes at 4°C to pellet the RNA.

825 µl ice-cold 100% ethanol

-20 °C

00:30:00

CENTRIFUGE
max. speed

00:15:00

4 °C

6 Carefully remove the supernatant. Wash the pellet using 300µl of 70% ethanol and dry.

300 µl 70% ethanol

7 Resuspend the pellet.