ab323459 – RNA Fragmentation Reagent Kit

For fragmentation of isolated RNA and purified mRNA for downstream processes including RNA-seq and next generation sequencing.

For overview, typical data and additional information please visit: www.abcam.com(use www.abcam.cn for China, or www.abcam.co.jp for Japan)

Storage and Stability: Store kit at -20 °C upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt. Aliquot components in working volumes before storing at the recommended temperature. Avoid repeated freeze-thaws of reagents.

Materials Supplied:

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
10X Fragmentation Buffer	400 µl	-20 °C	4 °C/-20 °C
10X Fragmentation Stop Solution	400 µl	-20 °C	4 °C/-20 °C

Materials Required, Not Supplied

PCR tubes
Thermal Cycler or dry heat block
Centrifuge
RNase-free filter tips and tubes (1.5 mL)
Purified RNA
3 M Sodium Acetate, pH 5.2
RNA Precipitation Carrier of choice
Nuclease-Free Water
Ethanol (Anhydrous 200 proof, Molecular Biology Grade)

Reagent Preparation: Briefly centrifuge small vials at low speed prior to opening.

1. **10X Fragmentation Buffer:** Thaw before use.
2. **10X Fragmentation Stop Solution:** Thaw before use.

Sample Preparation:

Prepare a fresh isolation of RNA, total RNA, or purified mRNA using method of choice.

RNA Fragmentation

Freshly isolated total RNA or purified mRNA is recommended for the following process. Avoid multiple freeze thaws of RNA and store at -80 °C.

1. **Preheat thermocycler to 94 °C.**
2. **Set up a Fragmentation Reaction for each RNA sample:**
   Make the following reaction mix in an RNase-free 200 µl PCR tube. We recommend using 1000 ng of purified RNA per reaction.

Component	Amount / reaction (µL)
Purified RNA	1-18
10X Fragmentation Buffer	2
Nuclease-Free Water	Variable
Total volume	**20**

3. Incubate reaction in preheated thermocycler for 1-5 minutes at 94 °C.
   ΔNOTE Fragmentation time can be adjusted depending on desired sizes of fragments.
4. Add 2 µl of 10X Fragmentation Stop Solution and briefly spin samples using a table-top mini centrifuge.
5. **Immediately** transfer the tube to ice and hold for 2 minutes and when samples are not in use.
6. **Precipitation.** Bring the sample volume to 100 µl by adding 78 µl of Nuclease-Free Water.
7. Add 10 µL of 3 M Sodium Acetate and RNA Precipitation Carrier of choice. Mix and carefully transfer fragmented RNA to a 1.5 ml Nuclease-free microcentrifuge tube.
   ΔNOTE If multiple samples are being precipitated, Nuclease-Free water, RNA Precipitation additive, and RNA Precipitation Carrier can be combined into an appropriate master mix and added to each sample.
8. Add 250 µL of ice-cold 100%(vol/vol) Ethanol and mix well.
9. Precipitate for 2-hours to overnight at – 20 °C. Centrifuge at 15,000×g for 30 min at 4 °C in a pre-cooled centrifuge. Carefully remove and discard the supernatant.
   ΔNOTE Precipitation can be performed for 30 minutes at -80°C.
10. Wash pellet by adding 500 µL of 75% (vol/vol) Ethanol and centrifuge at 15,000×g for 5 min at 4 °C in a pre-cooled centrifuge. Remove supernatant. Repeat
    ΔNOTE Fragmented RNA in 75% Ethanol can be held 2 hours to overnight at -20 °C as a safe stopping point before resuming protocol.
11. Air-dry the pellets until transparent and dissolve them in 10 µL of Nuclease-Free Water or TE.

Data analysis

Determine the yield and size distribution of the Fragmented RNA Samples via nucleic acid electrophoresis methods such as SYBR safe gel stain or with an automated electrophoresis device (Agilent Bioanalyzer 2100 Pico Chip Series).

Helpful Tips:

- After centrifugation of precipitated RNA, pellets are very small. It is recommended to use a 200 µL pipette tip when aspirating the supernatant as to not disturb the pellet and compromise recovery.
- Once RNA pellets have dried, they become transparent and difficult to see. Be sure to dry the pellet at the bottom of the microcentrifuge tube and resuspend by pipetting 10-20 times.
- Avoid over-drying of the pellet as it can make resuspension more difficult (10 minutes recommended).
- RNA Precipitation Carriers, such as glycogen, can interfere with absorbance readings and ratios. If downstream applications require Nanodrop absorbance readings, it is recommended to precipitate a sample of the Precipitation Carrier at the same ratio as the fragmented samples, resuspend in 10 µL of Nuclease-Free water, and take an absorbance reading. Correct the background interference of the RNA Precipitation Carrier from the Fragmented samples by subtracting the absorbance measurement of the Carrier from the Sample readings.
- Only use 1.5-1.7 ml tubes during precipitation to obtain good pellets. 2 ml tubes tend to distort pellet.
- It may be helpful to avoid using silanized or low retention tubes.

FAQ:

How many RNA samples can I fragment with this kit?
This kit is sufficient for 200 reactions.

How long should I incubate the fragmentation reaction?
Incubation time of RNA fragments can be optimized for desired fragment size. RNA will fragment into shorter sizes if left to incubate longer (5 minutes). Fragmentation will also vary depending on RNA species and isolation methods. It is suggested to run proof-of-concept testing to determine the optimal incubation time for your specific RNA samples.

What kind of QC can be done to ensure my samples have reacted properly before I send samples for sequencing?
Gel electrophoresis: To show size distribution of nucleotides in RNA samples to qualify fragmentation.

Agilent 2100/4200 TapeStation/Bioanalyzer: To visualize the size and yield of purified RNA fragments.

Sequencing: Test specific sequence, show repeatability and control.

Technical Support

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