

rtStar™ tRF&tiRNA Pretreatment Kit

Cat#: AS-FS-005

Instruction Manual version 1.0

Product summary

Product description

tRNA derived fragments (tRF) and tRNA halves (tiRNA) are heavily decorated by RNA modifications. rtStar™ tRF&tiRNA Pretreatment Kit is designed to remove the modifications that interfere with small RNA cDNA library construction of qPCR. These modifications include terminal modifications that block adaptor ligation to the RNA ends and internal methylations that hinder reverse transcription for cDNA synthesis. The kit does the following treatments:

- 3'-aminoacyl (charged) deacylation to 3'-OH for 3' adaptor ligation;
- 3'-cP (2',3'-cyclic phosphate) removal to 3'-OH for 3' adaptor ligation;
- 5'-OH (hydroxyl group) phosphorylation to 5'-P for 5'-adaptor ligation;
- m1A, m1G, and m3C demethylation for efficient reverse transcription.

The pretreated RNAs can be used for qPCR. The kit protocol is optimized for higher yields and quality of the treated RNA.

Kit components

The kit is sufficient for up to 12 reactions. The recommended starting material amounts can vary from 1 to 5 µg total RNA.

Kit component	Amount	Storage	Shipping
Deacylation Reaction Buffer (5×)	40 µL	-20°C	Dry ice
Deacylation Stop Buffer	250 µL	-20°C	Dry ice
RNase Inhibitor	25 µL	-20°C	Dry ice
Terminal Enzyme Reaction Buffer (10×)	65 µL	-20°C	Dry ice
10 mM ATP	65 µL	-20°C	Dry ice
Terminal Enzyme Mix	13 µL	-20°C	Dry ice
Demethylation Reaction Buffer (5×)	130 µL	-20°C	Dry ice
Demethylase	65 µL	-20°C	Dry ice
Demethylation Stop Buffer (5×)	130 µL	-20°C	Dry ice
Nuclease-free Water	1 mL	-20°C	Dry ice

Additional required materials

- RNase-free 200 µL PCR tubes
- Thermal cycler
- Microcentrifuge for 200 µL tubes
- Pipettors and tips

Protocol

3'-terminal Deacylation

1. Combine the reagents per deacylation reaction according to the following table in the order shown:

Input RNA	≤5 µg
Deacylation Reaction Buffer (5×)	3 µL
RNase Inhibitor	1 µL
Nuclease-free Water	x µL
Total volume per reaction	15 µL

2. Mix by vortexing and incubate at 37°C for 40 minutes.
3. Add 19 µL Deacylation Stop Buffer, mix by vortexing. Incubate at room temperature for 5 min.

3'-cP Removal and 5'-P Addition

4. Add the following reaction components on ice in the order given:

Terminal Enzyme Reaction Buffer (10×)	5 µL
10 mM ATP	5 µL
Terminal Enzyme Mix	3 U (1 µL)
Nuclease-free water	5 µL
Total reaction volume	50 µL

5. Incubate at 37°C for 40 minutes.
6. Inactivate the Terminal Enzyme by incubating at 70°C for 5 minutes.
7. Purify the RNA by phenol-chloroform extraction and ethanol precipitation.

Note: Column- or magnetic beads-based RNA purification methods can be used for Step 7. We highly recommend

Seq-Star™ RNAClean and smallEnrich Beads (Arraystar, Cat# AS-MB-009), which produces optimal results for this kit.

precipitation.

Note: Column- or beads-based RNA purification methods can be used for Step 11. We highly recommend Seq-Star™ RNAClean and smallEnrich Beads (Arraystar, Cat# AS-MB-009), which produces optimal results for this kit.

Demethylation

8. Prepare reagents before their immediate use: Remove Demethylase from the freezer, mix by flicking the tube (do not vortex). Briefly spin down the content and place on ice.
9. Set up demethylation mix: Combine the reagents per demethylation reaction according to the following table in the order shown. To account for pipetting losses, 10% excess for all the reagents is recommended for calculating multiple samples.

Nuclease-free water	x μ L
Demethylation Reaction Buffer (5 \times)	10 μ L
Demethylase	5 μ L
RNase Inhibitor	1 μ L
Input RNA	\leq 5 μ g
Total volume per reaction	50 μL

10. Perform demethylation reaction: Incubate the mix at 37°C for 2 h. Add 40 μ L Nuclease-free Water and then 10 μ L Demethylation Stop Buffer (5 \times) to terminate the reaction.
11. Purify the RNA by phenol-chloroform extraction and ethanol

