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rtStar™ tRF&tiRNA Pretreatment Kit

Cat#: AS-FS-005

Instruction Manual version 2.0

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 synthesis for 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'-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 then be used in cDNA synthesis for qPCR. The kit protocol is optimized for higher yields and better quality of the pretreated RNA. The condition in the RNA demethylation step may cause some RNA degradation and loss, but with negligible effects on the qPCR results for small RNA fractions including tRFs & tiRNAs.

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
RNase Inhibitor	36 uL	-20°C	Dry ice
10×Terminal Enzyme Reaction Buffer	36 uL	-20°C	Dry ice
10 mM ATP	36 uL	-20°C	Dry ice
Terminal Enzyme Mix	12 uL	-20°C	Dry ice
DRB-C1	100 uL	-20°C	Dry ice
DRB-C2	100 uL	-20°C	Dry ice
DRB-C3	100 uL	-20°C	Dry ice
DRB-C4	100 uL	-20°C	Dry ice
DRB-C5	100 uL	-20°C	Dry ice
Demethylase	60 uL	-20°C	Dry ice
Nuclease-free water	5 mL	-20°C	Dry ice

Additional required materials

- RNase-free 200 uL PCR tubes
- Microcentrifuge for 200 uL tubes
- Isopropanol
- Phenol, chloroform
- Thermal cycler
- Pipettors and tips
- 75% ethanol

Protocol

3'-cP Removal and 5'-P Phosphorylation

1. Gently thaw all of the kit components except for the Demethylase. Mix by brief vortexing, spin down all reagents and place on ice immediately.
2. Per reaction, combine the reagents according to the following table in the same order as shown:

Input RNA	⩽5 ug
RNase Inhibitor	1 uL
Terminal Enzyme Mix	1 uL
10× Terminal Enzyme Reaction Buffer	3 uL
10 mM ATP	3 uL
Nuclease-free water	x uL
Total reaction volume	**30 uL**

3. Vortex briefly to mix, and spin down. Incubate at 37°C for 30 min.
4. Orderly add 170 uL Nuclease-free water and 200 uL phenol:chloroform to terminate the reaction and perform the RNA purification procedure below immediately.
5. RNA purification
   1) Mix well the tube in **Step 4** by inverting. Incubate at room temperature for 10 min. Centrifuge at 12,000 rpm for 10 min.
   2) Carefully transfer the top layer by a micropipette to a RNase-free tube. Discard the bottom liquid phase to waste.
   3) Precipitate the RNA from the aqueous phase by mixing with an equal volume of Isopropanol. Vortex the mixture and place the tube at -80°C for 30 min. Centrifuge at 12,000 rpm for 10 min.

4) Remove the supernatant from the tube, leaving only the RNA pellet.

5) Add 1 ml 75% ethanol (in Nuclease-free Water) to wash the RNA pellet. Mix well by inverting.

6) Centrifuge the tube at 7,500 rpm for 5 min at 4°C. Discard the wash.

7) Air dry the RNA pellet for 5–10 min or by vacuum under centrifugation.

8) Fully dissolve the RNA pellet in 30 uL Nuclease-free water by incubating at 55–60°C for 10–15 min.

Note: *Column- or bead-based RNA purification method can be used for Step 5. We highly recommend Seq-Star™ RNAClean and smallEnrich Beads (Arraystar, Cat# AS-MB-009), which produces optimal results for this kit.*

Demethylation

6. Prepare fresh Demethylation Reaction Buffer (4×) by combine the DRB-C1, C2, C3, C4, C5 with 1:1:1:1:1 volume and keep on ice. The total required volume of Demethylation Reaction Buffer (4×) is 1.1 × 25 × number of samples (uL).

   Note: *Prepare fresh Demethylation Reaction Buffer (4×) each time before use.*

7. Remove Demethylase from the freezer, mix by flicking the tube (do not vortex). Briefly spin down the content and place on ice.

8. Set Up Demethylation Master Mix

   Per demethylation reaction, combine the reagents according to the following table in the same order as shown. To account for pipetting losses, 10% excess for all the reagents is recommended for calculating multiple samples.

Demethylation Reaction Buffer (4×)	25 uL
Demethylase	5 uL
RNase Inhibitor	2 uL
Nuclease-free water	38 uL
Total volume per reaction	**70 uL**

9. Perform demethylation reaction

   1) Add Demethylation Master Mix to the tube from **Step 5**.

   2) Vortex briefly to mix, spin down.

   3) Incubate at 25°C for 2 hr.

   4) Orderly add 100 uL Nuclease-free water and 200 uL phenol:chloroform to terminate the reaction and perform the RNA purification procedure below immediately.

10. RNA purification

    9) Mix well the tube in **Step 9** by inverting. Incubate at room temperature for 10 min. Centrifuge at 12,000 rpm for 10 min.

    10) Carefully transfer the top layer by a micropipette to a RNase-free tube. Discard the bottom liquid phase to waste.

    11) Precipitate the RNA from the aqueous phase by mixing with an equal volume of Isopropanol. Vortex the mixture and place the tube at -80°C for 30 min. Centrifuge at 12,000 rpm for 10 min.

    12) Remove the supernatant from the tube, leaving only the RNA pellet.

    13) Add 1 ml 75% ethanol (in Nuclease-free Water) to wash the RNA pellet. Mix well by inverting.

    14) Centrifuge the tube at 7,500 rpm for 5 min at 4°C. Discard the wash.

    15) Air dry the RNA pellet for 5–10 min or by vacuum under centrifugation.

    16) Fully dissolve the RNA pellet in 11 uL Nuclease-free water by incubating at 55–60°C for 10–15 min.

Note: *Column- or bead-based RNA purification method can be used for Step 10. We highly recommend Seq-Star™ RNAClean and smallEnrich Beads (Arraystar, Cat# AS-MB-009), which produces optimal results for this kit.*

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