ABclonal rRNA Depletion Module (H/M/R)

RK20348



Instruction Manual

Version: N19H20V1.2

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1. Overview

rRNA Depletion Module (H/M/R) is specifically designed for total RNA samples from human, mouse, and rat. It effectively removes cytoplasmic rRNA (including 5S rRNA, 5.8S rRNA, 18S rRNA, and 28S rRNA) and mitochondrial rRNA (including 12S rRNA and 16S rRNA) from total RNA. the depleted RNA can be used for whole RNA-seq library construction or other rRNA depletion analyses.

This module is compatible with both intact total RNA samples and degraded RNA samples, including FFPE RNA. It requires an input of 10 ng to 1 μg total RNA.

All reagents are subject to strict quality control. Each batch of kits is validated through library construction and sequencing, ensuring consistent and stable performance.

2. Kit Compents

Tube Color & Name	8 RXN	24 RXN	96 RXN
rRNA Probe Mix(H/M/R)	8 μL	24 μL	96 μL
Probe Hybridization Buffer	16 μL	48 μL	192 μL
RNase H	16 μL	48 μL	192 μL
10X RNase H Buffer	16 μL	48 μL	192 μL
DNase I	20 μL	60 μL	240 μL
10X DNase I Buffer	40 μL	120 μL	480 μL

3. Storage

All components should be strored at-20℃.

4. Additional Materials

4.1 Agencourt RNAClean XP beads (Beckman Coulter Inc., catalog number A63987)

4.2 80% Ethanol (freshly prepared)

4.3 Magnetic stand

4.4 PCR machine, etc.

5. Operational Precautions

5.1 During the RNA handling, wear masks and gloves, place RNA samples on ice, and proceed to the next experiment as quickly as possible to minimize RNA degradation.

5.2 Remove the magnetic beads from storage at least 30 minutes before use and allow them to equilibrate at room temperature. During the purification process, ensure that the alcohol has completely evaporated before adding Nuclease-free Water for washing. If the magnetic beads change color from bright brown to matte brown, it indicates either incomplete alcohol evaporation or over-drying (cracking), both of which will affect the efficiency of nucleic acid recovery.

5.3 Enzyme solutions in the kit are sensitive to temperature. Keep them on ice or in a low-temperature container during use, and return them to -20°C strorage immediately after use.

6. Protocols

6.1 rRNA Hybridization

6.1.1 Dilute 10-1000 ng of total RNA with nuclease-free water to a final volume of 12 μL and place the samples on ice.

6.1.2 Thaw the Probe Hybridization buffer on ice and prepare the probe hybridization mix as shown in Table 1 below.

Table 1. Hybridization Mix Setup

Components	Volume (per sample)
Probe Hybridization Buffer	2 μL
rRNA Probe Mix (H/M/R)	1 μL
Total Volume	3 μL

6.1.3 Add 3 μL of hybridization mix to 12 μL of diluted RNA, and mix thoroughly by pipetting up and down several times.

6.1.4 Briefly spin down the samples and place them on ice.

6.1.5 Incubate the samples in a thermocycler following the rRNA hybridization program shown in Table 2 below. (Set the heated lid to 105°C).

Table 2. rRNA Hybridization Condition

Temperature	Time
95 °C	2 min
95-22 °C	0.1°C/sec (continuously)
22 °C	5 min

6.1.6 After the hybridization incubation, place the samples on ice and proceed immediately to RNase H digestion.

6.2 RNase H Digestion

6.2.1 Remove the 10X RNase H Buffer from -20℃ storage, thaw on ice, and prepare the RNase H digestion mix as shown in Table 3 below.

Table 3. RNase H Digestion Mix Setup

Components	Volume (per sample)
10× RNase H Buffer	2 μL
RNase H	2 μL
Nuclease-free H_2O	1 μL
Total Volume	5 μL

6.2.2 Add 5 μL of the RNase H digestion mix to the hybridized RNA samples (from step 1.1.6) and mix thoroughly by pipetting.

6.2.3 Briefly spin down the samples and place them on ice.

6.2.4 Incubate the samples in a thermocycler following the RNase H digestion program shown in Table 4 below. (Set the heated lid to ≥45℃).

Table 4. RNase H Digestion Condition

Temperature	Time
37 ℃	30 min

6.2.5 Place the samples on ice and proceed immediately to DNase I digestion.

6.3 DNase I Digestion

6.3.1 Remove the 10X DNase I Buffer from -20℃ storage, thaw on ice, and prepare the DNase I digestion mix as shown in Table 5 below.

Table 5. DNase I Digestion Mix Setup

Components	Volume (per sample)
10× DNase I Buffer	5 μL
DNase I	2.5 μL
Nuclease-free H2O	22.5 μL
Total Volume	30 μL

6.3.2 Add 30 μL of the DNase I digestion mix to the RNase H digested samples (from step 6.2.5) and mix thoroughly by pipetting.

6.3.3 Incubate the samples in a thermocycler following the DNase I digestion program shown in Table 6 below. (Set the heated lid to ≥45℃).

Table 6. DNase I Digestion Condition

Temperature	Time
37 ℃	30 min

6.3.4 After digestion, place the sample on ice and proceed immediately to RNA purification.

6.4 rRNA-depleted RNA Purification

6.4.1 Remove the Agencourt™ RNAclean XP beads from 2-8 ℃ storage and resuspend by vortexing.

6.4.2 Add 110 μL (2.2X) of resuspended beads to the DNase I digestion reaction (~50 μL). Mix thoroughly by pipetting.

6.4.3 Incubate the samples on ice for 15 minutes.

6.4.4 Pellet the beads on a magnetic stand for 5 minutes and carefully remove the supernatant.

6.4.5 Keep tube with the beads on a magnetic stand and add 200 μL of 80% ethanol to wash the beads.

6.4.6 Incubate the samples at RT for 30 seconds and then carefully remove the supernatant.

6.4.7 Repeat step 6.4.5 and 6.4.6 for a total of two washes.

6.4.8 Air dry the beads on a magnetic stand for 2 minutes.

6.4.9 Resuspend the beads in 7 μL of Nuclease-free Water and mix thoroughly by pipetting.

6.4.10 Incubate at RT for 2 minutes.

6.4.11 Pellet the beads on a magnetic stand for 1 minute, then carefully transfer 5 μL of the supernatant to a new tube.

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