ABclonal rRNA Depletion Module (H/M/R)

(RK20348)

Instruction Manual

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The Kit Includes

Store at –20°C.

Probe Hybridization Buffer

rRNA Probe Mix(H/M/R)

RNase H

10× RNase H Buffer

DNase I

10× DNase I Buffer

Protocol

1. rRNA Depletion

1.1 rRNA hybridization.

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

1.1.2 Thaw the Probe Hybridization buffer on ice and prepare the probe hybridization mix as the 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

1.1.3 Add 3 µl of hybridization mix to 12 µl of the diluted RNA, and mix thoroughly by pipetting up and down several times.

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1.1.4 Spin down briefly and place the samples on ice.

1.1.5 Incubate the samples in a thermocycler and carry out the rRNA hybridization program according to the Table 2. below. (A heated lid is set to 105°C).

Table 2. rRNA Hybridization Condition

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

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

1.2 RNase H digestion.

1.2.1 Take the 10X RNase H Buffer from -20℃ storage, thaw on ice and prepare the RNase H digestion mix as the 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

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

1.2.3 Spin down briefly and place the samples on ice.

1.2.4 Incubate the samples in a thermocycler and carry out the RNase H

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digestion program according to the Table 4. below. (A heated lid is set to ≥45°C).

Table 4. RNase H Digestion Condition

Temperature	Time
37 ℃	30 min

1.2.5 Place the samples on ice and process to DNase I digestion immediately.

1.3 DNase I digestion

1.3.1 Take the 10X DNase I Buffer from -20℃ storage, thaw on ice and prepare the DNase I digestion mix as the 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 H_2O	22.5 μl
Total Volume	30 μl

1.3.2 Add 30 μL of DNase I digestion mix to the RNase H digested samples (step 1.2.5) and mix thoroughly by pipetting.

1.3.3 Incubate the samples in a thermocycler and carry out the DNase I digestion program according to the Table 6. below. (A heated lid is set to ≥45°C).

Table 6. DNase I Digestion Condition

Temperature	Time
37 ℃	30 min

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1.3.4 After digestion, place the sample on ice and process to RNA purification immediately.

1.4 rRNA-depleted RNA purification

1.4.1 Take the Agencourt™ RNAclean XP beads from 2-8 ℃ storage, resuspend by vortexing.

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

1.4.3 Incubate the samples on ice for 15 minutes.

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

1.4.5 Keep tube with the beads on a magnetic stand and add 200 µl of 80% ethanol to the tube.

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

1.4.7 Repeat step 1.4.5 and 1.4.6 for a total of two washes.

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

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

1.4.10 Incubate at RT for 2 minutes.

1.4.11 Pellet the beads on a magnetic stand for 1 minute, then carefully take 5 µl of supernatant samples to a new tube.

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