*Trans*NGS® rRNA Depletion Kit (Bacteria)

Cat. No. KD201

Storage: at -20°C for one year

Description

*Trans*NGS® rRNA Depletion Kit (Bacteria) depletes ribosomal RNA (5S rRNA, 16S rRNA, and 23S rRNA) from bacterial total RNA by RNase H digestion, while retains mRNA and other non-coding RNA. The final products can be used for RNA-seq, random-primed cDNA synthesis or other applications.

Highlights

- Efficient depletion of rRNA: up to 99% removal rat
- Simple workflow: 1.5 hours to complete

Sample Requirement

- RNA from Gram-positive or Gram-negative bacteria.
- 200 ng - 2 µg of total RNA from bacteria.
- Intact or partially degraded RNA.

Kit Contents

Component	KD201-11 (12 rxns)	KD201-03 (96 rxns)
rRNA Probe (Bac)	24 µl	192 µl
E.coli RNase H	24 µl	192 µl
DNase I	60 µl	480 µl
DB Enzyme Mix	12 µl	96 µl
5×BD Reaction Buffer	48 µl	384 µl
10×RNase H Reaction Buffer	30 µl	240 µl
10×DNase I Reaction Buffer	60 µl	480 µl
RNase-free Water	1 ml	4×1 ml

Procedures

Materials prepared by users: magnetic stand, freshly prepared 80% ethanol (using RNase-free water) and RNA purification beads (*MagicPure*® RNA beads-EC501 is recommended).

1. Probe hybridization to RNA

(1) Place a RNase-free PCR tube on ice, and add the following components

Component	Volume
Total RNA	X µl (200 ng~2 µg)
rRNA Probe (Bac)	2 µl
BD Enzyme Mix	1 µl
5×BD Reaction Buffer	4 µl
RNase-free Water	To 20 µl

(2) Pipette up and down to mix well. Briefly spin down the tube in a microcentrifuge if there is liquid on the tube wall.

(3) Incubate the tube in a thermocycler and incubate at 50 °C for 15 minutes. (The lid temperature is set to 55 °C).

(4) Briefly spin down the tube in a microcentrifuge and place on ice. Proceed immediately to the next step.

2. RNase H digestion

(1) Add the following components to a RNase-free PCR tube on ice

Component	Volume
Products from the previous step	20 µl
10×RNase H Reaction Buffer	2.5 µl
E.coli RNase H	2 µl
RNase-free Water	0.5 µl
Total volume	25 µl

(2) Pipette up and down to mix well. Briefly spin down the tube in a microcentrifuge if there is liquid on the tube wall.
(3) Incubate the tube in a thermocycler and incubate at 37 °C for 15 minutes. (The lid temperature is set to 40 °C)
(4) Place the tube on ice and proceed immediately to the next step.
3. DNase I digestion
(1) Add the following components to a RNase-free PCR tube on ice

Component	Volume
Products from the previous step	25 µl
10×RNase H Reaction Buffer	5 µl
DNase I	5 µl
RNase-free Water	15 µl
Total volume	50 µl

(2) Pipette up and down to mix well. Briefly spin down the tube in a microcentrifuge if there is liquid on the tube wall.
(3) Incubate the tube in a thermocycler and incubate at 37 °C for 15 minutes. (The lid temperature is set to 40 °C)
(4) Place the tube on ice and proceed immediately to the next step.
4. RNA purification with beads
(1) Transfer all the products from the previous step to a new 1.5 ml RNase-free microcentrifuge tube, add 110 µL (2.2x) RNA purification beads (TransGen *MagicPure*® RNA beads-EC501 is recommended), and pipette up and down to mix well.
(2) Incubate on ice for 15 minutes.
(3) Place the tube on a magnetic stand and incubate at room temperature until the solution turns clear (about 5 minutes), with the beads attached to the tube wall close to the magnetic stand completely. Discard the supernatant.
Note: If there is any liquid on the tube wall, briefly spin down the tube in a microcentrifuge before placing it on the magnetic stand to make sure all the magnetic beads have been pulled to the magnet. Be careful not to pipette the beads when discarding the supernatant, which will affect the final yield.
(4) Keep the 1.5 ml tube on the magnetic stand. Add 200 µl of freshly prepared 80% ethanol (prepared with RNase-free Water), and incubate at room temperature for 30 seconds. Do not pipette. Discard the supernatant.
Note: Do use freshly-prepared ethanol. Otherwise it will affect the experimental result.
(5) Repeat step (4) once.
(6) Keep the 1.5 ml tube on the magnetic stand, and air dry the beads at room temperature until the beads are about to appear imminent cracks (about 5 minutes).
(7) Remove the tube from the magnetic stand and add 12 µl of RNase-free Water to elute the RNA. Mix well by pipetting up and down or vortexing. Then incubate at room temperature for 2 minutes.
(8) Place the tube on the magnetic stand and incubate for about 2 minutes at room temperature until the solution is clear, to ensure all the magnetic beads are arranged on the tube wall near the magnet.
Note: Spin down the tube briefly in a microcentrifuge before placing it on the magnetic stand if there is liquid on the tube wall. Prolong incubation to 5 minutes if necessary to make sure that all the beads have been pulled to the magnet.
(9) Transfer 10 µl of eluent to a new RNase-free tube and perform downstream experiments such as NGS library construction on ice, or store at -80 °C.

Notes

- Avoid RNase contamination.
- RNA samples should be free of salts (such as Mg^{2+} or guanidinium salts) or organics (such as phenol or ethanol).
- The yield of non-ribosomal RNA depends on quality of the input RNA, starting rRNA content of the sample and the purification method after rRNA removal. Generally the recovery rate is 3% - 10%.

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