*Trans*NGS® rRNA Depletion Kit (Human/Mouse/Rat)

Please read the manual carefully before use.

Cat. No. KD101

Storage: at -20℃ for one year

Description

*Trans*NGS® rRNA Depletion Kit (Human/Mouse/Rat) depletes ribosomal RNA from 100 ng-1 μg human/mouse/rat total RNA by RNase H digestion, while retains mRNA and other non-coding RNA. The depleted ribosomal RNA contains both cytoplasmic ribosomal RNA (5S rRNA, 5.8S rRNA, 18S rRNA and 28S rRNA) and mitochodrial ribosomal RNA (12S rRNA and 16S rRNA). The resulting rRNA-depleted RNA is suitable for RNA-Seq, random-primed cDNA synthesis, or other downstream RNA analysis applications.

Features

• The kit can remove up to 99% ribosomal RNA from human/mouse/rat total RNA.

• Control qPCR Primer Sets are provided to monitor the depletion efficiency of ribosomal RNA and the retention rate of non-ribosomal RNA.

Applications

• Human/ Mouse/ Rat total RNA samples (100 ng-1 μg).

• This kit is suitable for both intact and partially degraded RNA (e.g. FFPE RNA).

Kit Contents

Component		KD101-11 (12 rxns)	KD101-03 (96 rxns)
rRNA Probe (H/M/R)		24 μl	192 μl
E.coli RNase H		12 μl	96 μl
DNase I		60 μl	480 μl
5×Hybridization Buffer		36 μl	288 μl
10×RNase H Reaction Buffer		24 μl	192 μl
10×DNase I Reaction Buffer		60 μl	480 μl
RNase-free Water		1 ml	8×1 ml
Control qPCR Primer Sets	Ribosomal RNA Primer Mix	24 μl	192 μl
	Non-ribosomal RNA Primer Mix	24 μl	192 μl

Procedures

Reagents not included in the kit: magnetic stand, freshly prepared 80% ethanol (made with RNase-free water), RNA beads

1. Hybridize the Probes to the RNA

(1) Place an RNase-free tube on ice and add the components as follows.

Component	Volume
Total RNA	x μl (10 ng-1 μg)
rRNA Probe(H/M/R)	2 μl
5× Hybridization Buffer	3 μl
RNase-free Water	to 15 μl

(2) Mix by pipetting up and down. Spin down briefly if there is liquid on the wall.

(3) Place the tube in a thermal cycler, and run the following program.

95℃	2 min
95→22℃	0.1℃/sec, takes approximately 12 min
22℃	5 min

(Please note that the cooling process must be slow.)

(4) Spin down briefly and place on ice. Proceed immediately to the next step.

2. RNase H Digestion

(1) Add the following components on ice.

Component	Volume
Reaction product of hybridization	15 µl
10× RNase H Reaction Buffer	2 µl
E.coli RNase H	1 µl
RNase-free Water	2 µl
Total volume	20 µl

(2) Mix by pipetting up and down. Spin down briefly if there is liquid on the wall.

(3) Place the tube in a thermo cycler (with heated lid set at 40°C) and incubate at 37°C for 15 minutes.

(4) Place the tube on ice. Proceed immediately to the next step.

3. DNase I Digestion

(1) Add the following components on ice.

Component	Volume
Reaction product of RNase H Digestion	20 µl
10× DNase I Reaction Buffer	5 µl
DNase I	5 µl
RNase-free Water	20 µl
Total volume	50 µl

(2) Mix by pipetting up and down. Spin down briefly if there is liquid on the wall.

(3) Place the tube in a thermo cycler (with heated lid set at 40°C) and incubate at 37°C for 15 minutes.

(4) Place the tube on ice. Proceed immediately to the next step.

4. RNA Purification Using RNA beads

(1) Transfer the 50 µl reaction product to a new 1.5 ml RNase-free centrifuge tube. Add 110 µl (2.2×) RNA Beads to the reaction product (RNA Beads recommended: TransGen Biotech *MagicPure*® RNA Beads, Cat. No. EC501). Mix by pipetting up and down.
Note: Insufficient mixing will affect the results significantly.

(2) Incubate on ice for 15 minutes.

(3) Place the tube on the magnetic stand and incubate at room temperature, make sure the beads settle to the magnet completely. After the solution is clear (about 5 minutes), discard the supernatant.
Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Make sure the beads settle to the magnet completely. Be careful not to disturb the beads, otherwise will affect the final yield.

(4) Add 200 µl of freshly prepared 80% ethanol (made with RNase-free water) to the tube on the magnetic stand, do not pipet the beads. Incubate at room temperature for 30 seconds. Discard the supernatant.
Note: Be sure to use freshly prepared ethanol, otherwise it will affect the experimental results.

(5) Repeat step (4) once.

(6) Air dry the beads at room temperature until the beads have just cracked (about 5 minutes) while the tube is on the magnetic stand.
Note: Be sure to dry the beads, otherwise subsequent experiments will be affected. Do not heat to dry, otherwise the final yield will be affected.

(7) Remove the tube from the magnetic stand. Add 12 µl RNase-free Water. Mix the beads by pipetting or vortexing. Incubate at room temperature for 2 minutes.

(8) Place the tube on the magnetic stand. Incubate at room temperature until the solution is clear (about 2 minutes). Make sure the beads settle to the magnet completely.

Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Incubation time can be extended to 5 minutes at room temperature. Make sure the beads settle to the magnet completely.

(9) Carefully transfer 10 µl of the eluate to a new RNase-free tube. Place the product on ice and proceed with NGS library construction or other downstream application. Alternatively, the product can be stored at -80℃.

5. qRT-PCR Detection (Optional)

*Trans*NGS® rRNA Depletion Kit (Human/Mouse/Rat) provides a pair of ribosomal RNA qPCR primer (Ribosomal RNA Primer Mix) and a pair of non-ribosomal RNA qPCR primer (Non-ribosomal RNA Primer Mix). To detect the depletion efficiency of ribosomal RNA, qRT-PCR test with the RNA sample before and after rRNA depletion can be adopted.

To ensure the consistency, the control RNA without rRNA depletion (Step 1-3) needs to go through RNA purification (step 4). Before proceeding with RNA purification, to equilibrate the volume of RNA sample, add RNase-free Water to make a total volume of 50 µl.

Suggested Template Amount of qRT-PCR

Two-Step qRT-PCR: 2 µl RNA is used as template for first-strand cDNA synthesis. 2 µl first-strand cDNA is used as template for qPCR reaction.

One-Step qRT-PCR: 10× diluted RNA is used.

TransGen Biotech Two-Step qRT-PCR as an example

Samples: 1 µg human/mouse/rat total RNA

Reagents: *MagicPure*® RNA Beads (Cat. No. EC501), *TransScript*® First-Strand cDNA Synthesis SuperMix (Cat. No. AT301), *PerfectStart*® Green qPCR SuperMix (Cat. No. AQ601).

(1) qRT-PCR system and conditions

• First-Strand cDNA synthesis

Component	Volume
rRNA depleted/undepleted RNA	2 µl
Random Primer(N9)	1 µl
2×TS Reaction Mix	10 µl
TransScript® RT/RI Enzyme Mix	1 µl
RNase-free Water	6 µl
Total volume	20 µl

Reaction conditions

Incubate at 25℃ for 10 minutes. After that, incubate at 42℃ for 15 minutes. Then, incubate at 85℃for 5 seconds to inactivate *TransScript*® RT/RI.

• qPCR

Component	Volume
cDNA	2 µl
Ribosomal RNA Primer Mix/Non-ribosomal RNA Primer Mix	1 µl
2×*PerfectStart*® Green qPCR SuperMix	10 µl
Nuclease-free Water	7 µl
Total volume	20 µl

Reaction conditions

94℃ 30 sec

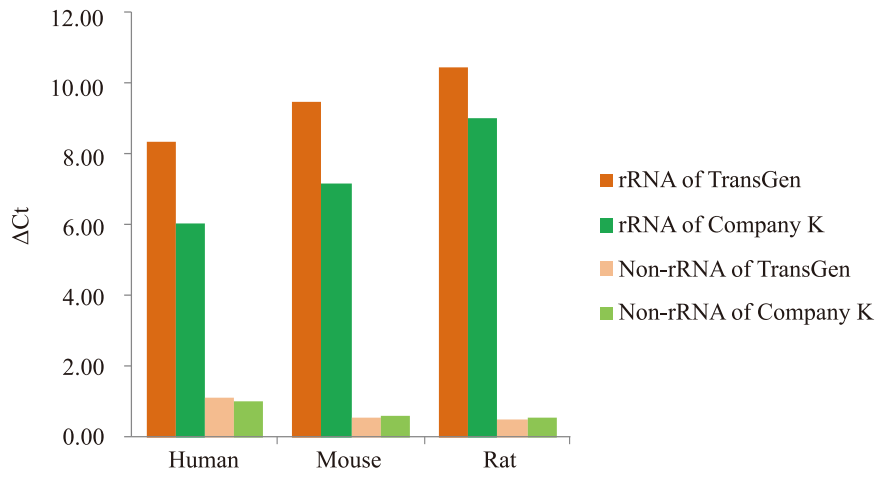
94℃ 5 sec

60℃ 30 sec

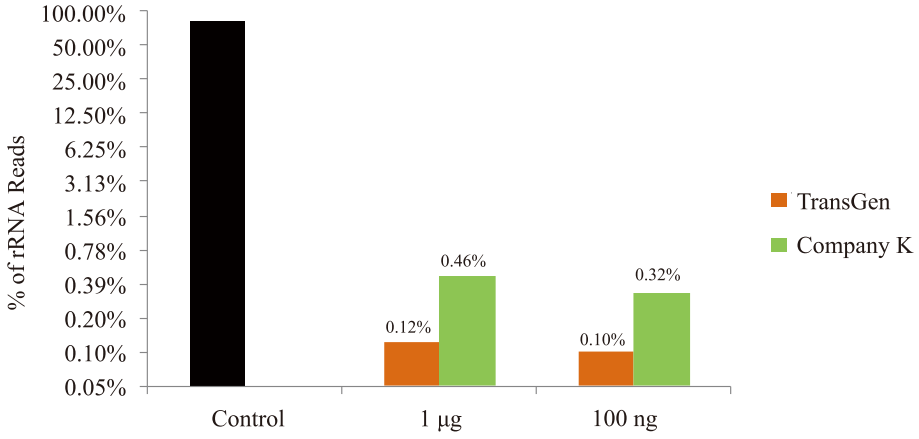
40 cycles

Dissociation Stage

(2) qRT-PCR result



Detection of Ct value changes before and after rRNA removal by qRT-PCR



rRNA percentage: 1 μg and 100 ng total RNA from HepG2 cells was treated using rRNA Depletion Kits of TransGen (KD101) and Company K. Sequencing reads were identified as ribosomal using Mirabait with human 18S, 28S, 5S, 5.8S, 12S and 16S ribosomal RNA sequences as baits.

Notes

- Avoid RNase contamination.
- The RNA samples should not contain any saline ion (e.g., Mg^{2+}, or guanidinium salts) or organic solvent (e.g., phenol and ethanol).
- The yield of non-ribosomal RNA depends on quality of the input RNA, the rRNA content of the sample, and the method used to purify the rRNA-depleted RNA. Typical recovery rate is 3%- 10%.

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Service telephone +86-10-57815020
Service email complaints@transgen.com