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

Cat. No. KD101

Storage: at -20°C for one year

Description

*Trans*NGS® rRNA Depletion Kit (Human/Mouse/Rat) depletes ribosomal RNA from human/mouse/rat total RNA by RNase H digestion, while retains messenger RNA and 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.

- 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.

Sample Requirement

- Human/ Mouse/ Rat total RNA samples (10 ng-1 μg).
- This kit is suitable for both intact and degraded RNA (e.g. FFPE RNA).

Kit Contents

Component		KD101-01 (6 rxns)	KD101-02 (24 rxns)	KD101-03 (96 rxns)
rRNA Probe (H/M/R)		12 μl	48 μl	192 μl
E.coli RNase H		6 μl	24 μl	96 μl
DNase I		30 μl	120 μl	480 μl
5×Hybridization Buffer		18 μl	72 μl	288 μl
10×RNase H Reaction Buffer		12 μl	48 μl	192 μl
10×DNase I Reaction Buffer		30 μl	120 μl	480 μl
RNase-free Water		500 μl	2×1 ml	8×1 ml
Control qPCR Primer Sets	Ribosomal RNA Primer Mix	12 μl	48 μl	192 μl
	Non-ribosomal RNA Primer Mix	12 μl	48 μl	192 μl

Procedures

Reagents supplied by user: 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)	1 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. Place samples in a thermocycler, and run the following program

95°C	2 min
95→22°C	0.1°C/sec, takes approximately 12 minutes
22°C	5 min

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

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

2. RNase H Digestion

(1) On ice, prepare RNase H digestion mix as follows

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 samples in a thermocycler (with lid at 40°C) and incubate at 37°C for 30 minutes.

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

3. DNase I Digestion

(1) On ice, prepare DNase I digestion mix as follows

Component	Volume
Reaction product of RNase H Digestion	20 µl
10×DNase I Reaction Buffer	5 µl
DNase I	1 µl
RNase-free Water	24 µl
Total volume	50 µl

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

(3) Place samples in a thermocycler (with lid at 40°C) and incubate at 37°C for 30 minutes.

(4) Place samples 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 EP 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 an appropriate magnetic stand to separate beads from the supernatant at room temperature. When 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 that RNA beads are settled to the magnet completely and not to be disturbed when discarding the supernatant. Discarding beads will result in reduced yield.

(4) With the EP tube still on the magnetic stand, add 200 µl of freshly prepared 80% ethanol (made with RNase-free water) and incubate at room temperature for 30 seconds without pipetting up and down. Carefully remove and discard the supernatant.

Note: Use freshly-prepared 80% ethanol; otherwise it may affect the result.

(5) Repeat previous step (4) once for a total of two washes.

(6) Air dry the beads for up to 5 minutes while the tube is on the magnetic stand with the lid open.

Note: Residual ethanol may influence the downstream reaction. Do not over dry or heat the beads, which may result in reduced yield.

(7) Remove the tube from the magnetic stand and elute RNA with 12 µl of RNase-free water. Mix well by pipetting up and down or vortexing. Then incubate at room temperature for 2 minutes.

(8) Put the tube back to the magnetic stand. Incubate for 2 minutes (or until the solution is clear).

Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Prolong incubation to 5 minutes if necessary to make sure that RNA beads are settled to the magnet completely.

(9) Transfer 10 µl of the supernatant to a new RNase-free tube. Place the sample on ice and proceed with NGS library construction or other downstream application. Alternatively, the sample can be stored at -80°C.

5. qRT-PCR Detection (Optional)

*Trans*NGS® rRNA Depletion Kit (Human/Mouse/Rat) provides a pair of ribosomal RNA primer (Ribosomal RNA Primer Mix) and a pair of non-ribosomal RNA 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 RNA sample 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 a total volume of 50 µl.

Suggested Template amount of qRT-PCR

Two-Step qRT-PCR: 2 µl RNA is used as template for of first-strand cDNA synthesize. 2 µl first-strand cDNA is use 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), *TransStart*® Tip Green qPCR SuperMix (Cat. No. AQ141).

(a) qRT-PCR reaction

• 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

Incubation

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

• qPCR

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

Thermal cycling conditions

94°C 30 sec

94°C 5 sec

60°C 30 sec ⌋ 40 cycles

Dissociation Stage

(b) qRT-PCR result

Control qPCR Primer Sets	RNA Template	Ct		
		Human	Mouse	Rat
Ribosomal RNA Primer Mix	rRNA depleted	12.2	11.4	11.0
	rRNA undepleted	19.2	19.6	20.7
Non-ribosomal RNA Primer Mix	rRNA depleted	15.3	16.7	17.5
	rRNA undepleted	16.5	18.2	18.8
Depletion efficiency of rRNA		99.2%	99.7%	99.9%

Notes

- Avoid contamination of RNase at all steps.
- 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 actual yield 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%.
- For RNA-seq purpose, we recommend using more 100 ng starting total RNA to increase library complexity and reduce sequencing duplication rates.

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