Magbio Small RNA Library Prep Kit for Illumina

Magbio # M322
24/96 reactions

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Magbio Small RNA Library Prep Kit for Illumina Includes:

The volumes provided are sufficient for preparation of up to 24/96 reactions (Magbio # M3221/M3222). All reagents should be stored at -20 ℃.

- (red) 3 ADT
- (red) RNA Ligase 1
- (red) RNA Ligation Buffer 1
- (red) Ligation Enhancer Mix
- (white) 5 ADT
- (white) RNA Ligase 2
- (white) RNA Ligation Buffer 2
- (green) RT Primer
- (yellow) RT buffer Mix
- (blue) RNase Inhibitor
- (gray) Reverse Transcriptase
- (pink) Primer-Universal
- (orange) HF DNA Polymerase
- (orange) 5×PCR Buffer
- (orange) dNTP (2.5 mM)
- (orange) Magbio Primer-Index 1
- (orange) Magbio Primer-Index 2
- (orange) Magbio Primer-Index 3
- (orange) Magbio Primer-Index 4
- (orange) Magbio Primer-Index 5
- (orange) Magbio Primer-Index 6
- (orange) Magbio Primer-Index 7
- (orange) Magbio Primer-Index 8
- (orange) Magbio Primer-Index 9
- (orange) Magbio Primer-Index 10
- (orange) Magbio Primer-Index 11
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- (orange) Magbio Primer-Index 23
- (orange) Magbio Primer-Index 24

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1. Ligate the 3´ Adaptor

1.1. Mix the following components in a sterile nuclease-free PCR tube:

Input RNA (1ug)	1–4 µl
● (red) 3 ADT	1 µl
Nuclease-Free Water	variable
Total volume	5 µl

1.2. Incubate in a preheated thermal cycler for 2 minutes at 70 °C.
Transfer tube to ice for 2 minutes.

1.3. Add the following Components:

● (red) RNA Ligation Buffer 1	1.8 µl
● (red) RNA Ligase 1	0.5 µl
● (red) Ligation Enhancer Mix	2.2 µl
● (blue) RNase Inhibitor	0.5 µl
Total volume	10 µl

Note: You have to add the Ligation Enhancer Mix individually.

1.4. Incubate for 2 hours at 37 °C in a thermal cycler, and place tube on ice.

1.5. Add 1 µl ● (green) RT Primer, incubate at 75 °C for 5 min, 37 °C for 30 min, 25 °C for 15 min; place the tube on ice.

2. Ligate the 5´Adaptor

2.1. Add the following components to the ligation mixture from Step 1.5 and mix well:

○ (white) RNA Ligation Buffer 2	1.4 µl
○ (white) RNA Ligase 2	1.0 µl
○ (white) 5 ADT	1.0 µl
● (blue) RNase Inhibitor	0.5 µl
Total volume	14.9 µl

2.2. Incubate for 2 hours at 37 °C in a thermal cycler, and place tube on ice.

3. Perform Reverse Transcription

3.1. Mix the following components in a sterile, nuclease-free tube:

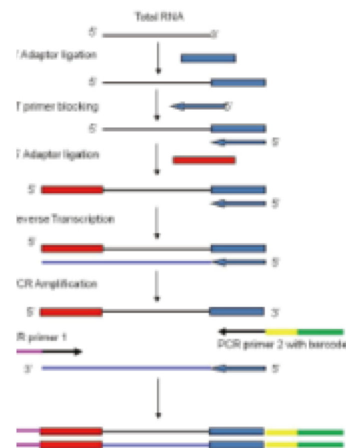
RNA Ligation Product	14.9µl
● (yellow) RT Buffer Mix	8 µl
● (gray) Reverse Transcriptase	1 µl
● (blue) RNase Inhibitor	1 µl
Total volume	24.9 µl

Overview

The Magbio Small RNA Library Prep Kit contains the adaptors, primers, enzymes and buffers required to convert small RNAs into indexed libraries for next-generation sequencing on the Illumina platform. The novel workflow has been optimized to minimize adaptor-dimers, while producing high-yield, high-diversity libraries. Each kit component must pass rigorous quality control standards, and each set of reagents is functionally validated together by construction and sequencing of a indexed small RNA libraries on the Illumina sequencing platform.

RNA Sample Quality

This kit was optimized using high quality human RNA (First Choice Human Brain Reference RNA from Life Technologies, Inc. #AM6050). High quality total RNA [RNA Integrity Number (RIN) > 7] should be used as starting material whenever possible. The quality and quantity of your sample should be assessed, for example by use of the Agilent 2100 Bioanalyzer, using an Agilent RNA 6000 Nano Chip.



Protocols

Please refer to revision history for a summary of protocol updates.
Libraries prepared by this method are compatible with Illumina paired-end flow cells.

Starting Material: 100 ng–1 µg Total RNA.

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3.2. Incubate for 1 hour at 50 °C, followed by 15 minutes at 70 °C, then hold at 4 °C in a thermal cycler.

3.3. Immediately proceed to PCR amplification.

Safe Stopping Point: If you do not plan to proceed immediately to PCR amplification. Samples can be safely stored at -15℃ to -25℃.

4. Perform PCR Amplification

4.1. Add the following components to the RT reaction mix from Step 4.2 and mix well:

RT Product	10 µl
● (orange) 5×PCR Buffer	10 µl
● (orange) HF DNA Polymerase	0.5 µl
● (orange) dNTP (2.5 mM)	5 µl
● (orange) Primer-Index	2 µl
● (pink) Primer-Universal	2 µl
Nuclease-Free Water	20.5 µl
Total volume	50 µl

Note: The Magbio Small RNA Library Prep Kit PCR primers, each with a different index. For each reaction, only one of the 24 PCR primer indices is used during the PCR step.

4.2. PCR Cycling conditions:

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	98 °C	30 sec	1
Denaturation	98 °C	10 sec	18*
Annealing	60 °C	30 sec	
Extension	72 °C	15 sec	
Final Extension	72 °C	7 min	1
Hold	4 °C	∞	

Amplification conditions may vary based on RNA input amount, tissue, and species. This protocol was optimized using 1 µg of total RNA from human brain and 18 PCR cycles.

5. QC Check and Size Selection using 6% PolyAcrylamide Gel

5.1. Purify the PCR amplified cDNA construct (50 µl) using a QIAQuick PCR Purification Kit.

IMPORTANT: Before eluting the DNA from the column, centrifuge the column with the lid of the spin column open for 5 minutes at 13,200 rpm. Centrifugation with the lid open ensures that no ethanol remains during DNA elution. It is important to dry the spin column membrane of any residual ethanol that may interfere with the correct loading of the sample on the PAGE gel.

5.2. Elute amplified DNA in 27.5 µl Nuclease-free Water.

5.3. Mix the purified PCR product (25 µl) with 5 µl of Gel Loading Dye, Blue (6X).
Note: Vortex the Gel Loading Dye, Blue throughly to mix well before using.

5.4. Load 5 µl of Quick-Load pBR322 DNA-MspI Digest in one well on the 6 % PAGE 10-well gel.

5.5. Load two wells with 15 µl each of mixed amplified cDNA construct and loading dye on the 6 % PAGE 10-well gel.

5.6. Run the gel for 1 hour at 120 V or until the blue dye reaches the bottom of the gel. Do not let the blue dye exit the gel.

5.7. Remove the gel from the apparatus and stain the gel with SYBR Gold nucleic acid gel stain in a clean container for 2–3 minutes and view the gel on a UV transiluminator (Figure 1).

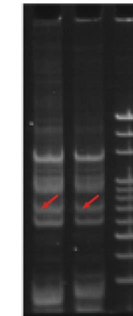

Figure 1: Shows typical results from Human Brain Total RNA libraries.

5.8. The 140 and 150 nucleotide bands correspond to adapter-ligated constructs derived from the 21 and 30 nucleotide RNA fragments, respectively. For miRNAs, isolate the bands corresponding to ~140 bp. For piRNAs, isolate the band corresponding to ~150 bp.

5.9. Place the two gel slices from the same sample in one 2 ml tube and crush the gel slices with the RNase-free Disposable Pellet Pestles and then soak in 500 µl DNA Gel Elution buffer (1X).

5.10. Rotate end-to-end for at least 2 hours at room temperature.

5.11. Transfer the eluate and the gel debris to the top of a gel filtration column.

5.12. Centrifuge the filter for 2 min at > 13,200 rpm.

5.13. Recover eluate and add 1 µl Linear Acrylamide, 25 µl 3M sodium acetate, pH 5.5 and 750 µl of 100 % ethanol,vortex well.

5.14. Precipitate in a dry ice/methanol bath or at –80 °C for at least 30 minutes.

5.15. Spin in a microcentrifuge > 14,000 × g for 30 minutes at 4 °C.

5.16. Remove the supernatant taking care not to disturb the pellet.

5.17. Wash the pellet with 80 % ethanol by vortexing vigorously.

5.18. Spin in a microcentrifuge > 14,000 ×g for 30 minutes at 4 °C.

5.19. Air dry pellet for up to 10 minutes at room temperature to remove residual ethanol.

5.20. Resuspend pellet in 12 µl TE Buffer.

5.21. Load 1 µl of the size selected purified library on a 2100 Bioanalyzer using a High Sensitivity DNA chip according to the manufacturer's instructions.

5.22. Check the size, purity, and concentration of the sample.

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