BioVendor R&D®

miRNA Isolation Kit Tissue

Kit Description

The *miRNA Isolation Kit* Tissue enables purification of 19-24 nucleotides miRNA, small RNA and less than 1000 nucleotides RNA from tissue samples. Based on optimized reagent buffer and silica membrane column, this kit is able to get high quality and purity of miRNA, which can be used in wide range of downstream application such as qPCR, Microarray and NGS. It provides a convenient and eco-friendly protocol without using phenol or chloroform for RNA purification.

Kit Content

	4rxn	50rxn	250rxn	
MR22 Column	4	50	250	pcs
Collection Tube (2ml)	12	150	750	pcs
Buffer RTL	1.6	20	100	ml
Buffer RCL1	0.36	4.5	22.5	ml
Buffer RCL2	0.12	1.5	7.5	ml
Buffer CRW1 (concentrated)	0.48	6	30	ml
Buffer CRW2 (concentrated)	0.96	12	60	ml
RNase-Free H2O	0.96	12	60	ml

Kit Storage

Upon arrival,
1. Please store **MR22 Column** at **4**℃ for long term storage.
2. Buffer, solvent and consumables, please store at 15-25℃.

Kit Preparation

1. Prepare Buffer CRW1
Add 4 volume of 100% EtOH into concentrated Buffer CRW1 to get Buffer CRW1.
After adding 100% EtOH, please tick the sticker on the bottle and close the cap tightly.
2. Prepare Buffer CRW2
Add 4 volume of 100% EtOH into concentrated Buffer CRW2 to get Buffer CRW2.
After adding 100% EtOH, please tick the sticker on the bottle and close the cap tightly.

General Protocol

1. Weight up to 25 mg of tissue sample or no more than 10 mg spleen tissue. Homogenize tissue sample with liquid nitrogen. Grind tissue sample thoroughly with liquid nitrogen by beads beater, tissue homogenizer or mortar & pestle. Please must grind tissue into fine powders, insufficient homogenization will lead to improper lysis and decrease the yield. Besides, avoid any thaw out during homogenization to keep the integrity of RNA.
2. Add 330µl Buffer RTL (add 1% β-mercaptoethanol freshly), vortex vigorously for 30 sec, brief spin down then incubate at 25°C (room temperature) for 5 min. Please must vortex vigorously in order to let Buffer RTL can lyse tissue powders thoroughly.
3. Centrifuge at 11,000 x g for 3 min. Transfer 250µl of clear supernatant to a new 1.5 ml micro-centrifuge tube.
4. Add 75µl Buffer RCL1. Pulse-vortexing for 10 sec , brief spin down then incubate at 25°C (room temperature) for 3 min.
5. Add 25µl Buffer RCL2, pulse-vortexing for 10 sec, brief spin down then incubate at 25°C (room temperature) for 1 min.
6. Centrifuge at 11,000 x g for 3 min.
7. Transfer 250µl clear supernatant to a new 1.5 ml micro-centrifuge tube, add 330µl of isopropanol, pulse-vortexing for 10 sec then briefly spin down. (If the volume of supernatant is less than 250µl, please measure the volume of lysate and add 1.3 volume of isopropanol.)

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8. Transfer all mixture to MR22 Column (with 2ml Collection Tube), incubate at 25°C (room temperature) for 2 min.
9. Centrifuge at 11,000 x g for 1 min. Change a new collection tube.
10. Add 700µl Buffer CRW1 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
11. (Optional) DNase I (not provided) on column digest can be performed in between these two wash step.
12. Add 700µl Buffer CRW2 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
13. Add 700µl Buffer CRW2 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
14. Change a new collection tube, centrifuge at 11,000 x g for 3 min.
15. Place the spin column into 1.5 ml micro-centrifuge tube, add 30-100µl RNase-Free H2Oand incubate at 25°C (room temperature) for 2 min.
16. Centrifuge at 11,000 x g for 1 min for elution.

FOR RESEARCH USE ONLY

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