Use 10 to 2.5ug of total RNA, depending on abundance. The optimal sample range is 1000ng to 1ug.

Using Ncode kit:

**PolyA tailing of miRNA(1ug of RNA):**

1. Based on quantity of total RNA, dilute a volume of 10mM ATP in 1mM Tris(pH 8) according to formula: atp dilution factor 5000/ (1000ng of RNA).
2. Prep mastermix:

|  |  |  |
| --- | --- | --- |
| Component | Vol for 1 reaction. |  |
| RNA | X (eg. 1ug) |  |
| 5x miRNA reaction Buffer | 5 ul |  |
| 25mM MnCl2 | 2.5 ul |  |
| Diluted ATP (step 1) | 1 |  |
| PolyA polymerase | 0.5 |  |
| DEPC-treated water | To reach 25ul |  |

1. Mix gently and spin down
2. Incubate tube in heat block for 15min at 37 degrees.
3. After incubation proceed to first strand synthesis.

**First Strand cDNA Synthesis.**

1. Add into RNase-free microcentrifuge tube.

|  |  |
| --- | --- |
| Component | Volume (ul) |
| PolyA’d RNA from above | 4 |
| Annealing buffer | 1 |
| Uni RT primer (25uM) | 3 |
| **Total volume** | **8** |

1. Incubate 65 degrees for 5minutes.
2. 4 degrees for 1 minute.
3. Add this:

|  |  |
| --- | --- |
|  | ul |
| 2x first strand reaction mix | 10 |
| Superscript enzyme mix | 2 |

1. Spin down
2. Into thermal cycler preheated to 50degrees for 50minutes.
3. Incubate at 85 degrees for 5 min to stop reaction
4. Chill. Either on ice or at 4degrees, store at -20 or proceed to pcr.

**qPCR using SYBR Green supermix (for 50ul reaction)**

1. Dilute cDNA 1:10 in DECP-treated water. Use 1ul of diluted cDNA per 10ul reaction.
2. Put samples of cDNA into 384 well plate.
3. Mastermix:

|  |  |  |
| --- | --- | --- |
| Comp | uL | Concentration |
| Plat. SYBR supermix | 5 | 1x |
| Forward primer | 0.2 | 200nM |
| Uni primer qPCR | 0.2 | 200nM |
| template | 1 | 1%v/v cDNA |
| DECP treated | Up to 10uL | - |

1. Spin plates, 3min, 300g.
2. Into qPCR machine.