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USER MANUAL NAW001

miRNA qPCR ASSAYS
powered by NAWGEN

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Our miRNA qPCR Assays were developed by miRNA experts at Nawgen to improve upon previously available qPCR assays, resulting in assays that are more specific, more sensitive, and less variable.

INCLUDED

- Reverse transcription primer
- Forward and reverse primers for each miRNA
- Reagents provide enough for 250 reactions

PRODUCT DESCRIPTION

MicroRNAs (miRNAs) are a family of small non-coding RNA molecules that downregulate the expression of their gene target. miRNAs are master regulators of many important biological processes including a variety of human diseases. One major challenge in miRNA research is to accurately and conveniently determine the expression level of miRNAs in various experimental systems. As deregulated expression of miRNAs often indicates functional deregulation, screening for miRNAs with altered expression profiles is likely to provide clues for the involvements of miRNAs in disease development.

Currently, several commercial products are available for PCR-based miRNA detection and quantitation. However, there are major design limitations associated with these available assays, with inadequate considerations for both assay sensitivity and specificity. To address these technical issues, we have developed a new real-time RT-PCR method, using a bioinformatics algorithm for miRNA primer design by incorporating multiple design filters to improve miRNA assay specificity, sensitivity and homogeneity (Wang 2009). This algorithm has incorporated multiple design features that have been proved to be important in our previous studies (Wang and Seed 2003). In addition, our new algorithm also includes novel primer features that are uniquely associated with miRNA assay design.

PERFORMING RT REACTIONS

Note: Nawgen microRNA qPCR assays are optimized to work with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems Catalog number 4368813 or 4368814).

1. Allow the 20X RT primers to thaw on ice.
2. Referring to the table below, calculate the volume of components needed to prepare the required number of reactions for a 2X master mix.

Component	Volume/Reaction (µl)
10x RT Buffer	2.0
25x dNTP (100 nM)	0.8
10x RT Master Mix	1.0
Multiscribe Reverse Transcriptase	1.0
Nuclease-free H2O	5.2
Total 2x Master Mix per Reaction	**10.0**

3. Place the 2X master mix on ice and mix gently.
4. Pipette 10 µL of 2x RT master mix into each PCR tube.
5. Pipette 10 µL of RNA sample into each tube, pipetting up and down two times to mix.
   Note: 1ng-1µg RNA can be added to each reaction (100ng recommended).
6. Vortex and then spin briefly to remove any air bubbles.
7. Run RT reaction at the following conditions using a thermal cycler:
   Note: These conditions are optimized for use with the High-Capacity cDNA Reverse Transcription Kit.

CANOPY BIOSCIENCES

	Step 1	Step 2	Step 3	Step 4
Temperature (°C)	25	37	85	4
Time (min)	20	60	5	∞

8. Keep the 1st strand cDNA on ice for immediate use, or store at -20° C for later use.

PERFORMING REAL-TIME PCR

Note: Nawgen microRNA qPCR assays are optimized to work with the Power SYBR Green Master Mix (2X) (Applied Biosystems Catalog Number 4367659) for Real-time PCR at a 20µl reaction volume.

1. Thaw the cDNA samples and PCR primers on ice, then vortex and centrifuge briefly.
2. Prepare the appropriate number of reactions in an optical PCR plate.

Component	Volume/Reaction (µl)
Power SYBR Green Master Mix (2X)	10
20x miRNA Primer Pair	1
cDNA template	1
Nuclease Free Water	8
Total	**20**

3. Seal the plate with an optical adhesive cover. Mix the components thoroughly, and then centrifuge the plate briefly to eliminate any air bubbles.
4. 4. Place the reaction plate in a real-time PCR instrument.
5. 5. Set the thermal cycling conditions as follows:

Note: These are optimized cycle conditions to be used with Power SYBR Green Master Mix. It may be necessary to alter Stage 1 incubation time depending on master mix selection. We highly recommend using a two-step system (Stage 2 & 3). Start real-time PCR data collection during stage 2.

Stage	Step	Temperature (°C)	Duration	Cycles
1	Polymerase Activation	95	10 min	1
2	Denaturation	95	15 sec	3
	Annealing	45	1 min	
	Extension	60	30 sec	
3	Denaturation	95	10 sec	35
	Anneal/Extend	60	30 sec	