NOYA Stem-Loop RT-qPCR kit
For miRNAs
Catalog number: N-1241
« Research Use Only »

Overview

Increase interest in the biological functions of small RNAs such as microRNAs (miRNAs) warrant convenient methods for quantification of these small RNA species. However, miRNAs are too short to accommodate an adequate primer and probe for any level of specific amplification using standard methods. Here we introduce a method for quantitative amplification of specific miRNAs whereby the target cDNAs is lengthened and design specific primer plus universal primer combine to ensure specificity at great sensitivity. First strand cDNA synthesis with a highly stable stem-loop primer lengthens the target from its original ~22 to >60 nts. Real time PCR (SYBR Green) amplification utilize a forward primer that includes extra 5´nts to adjust for an appropriate Tm, an universal reverse primer that is complementary to a sequence with RT stem–loop primer.

Kit Content

Specific RT stem-loop primer (inquire)
Universal reverse primer (inquire)
Forward primer (inquire)
NOYA Stem – Loop RT master mix
NOYA SYBR detect MMX

Other supplies (Required)

- Disposable gloves
- Precision pipette
- Aerosol resistant pipette tip
- Sterile 1.5ml tube
- Nuclease free water
- Plastic PCR tube or 96-well reaction plates
- Real Time PCR for quantitative PCR

Storage

Store the Kit at -20°C. Repeated freezing and thawing may reduce the sensitivity and should be avoided

Procedure

Set up the experimental reaction by adding in order the following components:

For 1 reaction	
Components	Volume/reaction
RT stem –loop master mix	6µl/rxn
RT specific Primer (10µM)	2µl/rxn
RT enzyme mix	0.5µl/rxn
RNA template (<1ug)	1.5µl
Total volume	10µl

For different volumes calculate all component proportionally

1. Pipette all components on ice or cool box. (Minimize exposure of the master mix to light). minimize pipetting error by avoiding very small volume (5 µl) and using calibrated pipette
2. Genteelly mix the reaction s avoiding bubble (do not vortexing)
3. Dispense appropriate volume into PCR tubes or plates

Thermal profile

Place the reaction in the instrument and run the appropriate PCR program below.

Step	Time	temp	additional comment
Initial step	30 min	16°C	Annealing step
Final step	30 min	40°C	cDNA synthesis step
inactivation*	3 min	95 °C	enzyme inactivation
Cycle number 1			

qPCR miRNA

Like other Syber Green real time PCR experiments. **Use the product of above procedure as template.**

Set up the experimental reaction by adding in order the following components:

For 1 reaction		
Components	Volume/reaction	final concentration

2X NOYA-SYBR detect	10 µl/rxn	1X
Universal primer (10 µM)	variable	0.1-0.5 µM
Forward primer (10 µM)	variable	0.1-0.5 µM
Enzyme	0.3µl	----
H2O	variable	
Template	variable *	
Total volume	20 µl	

*Template level is depend on its expression of miRNA but generally 2 µl is good starting point

Thermal profile

Place the reaction in the instrument and run the appropriate thermal program below. Although optimization of amplification protocol for each primer /template system is recommended, but following protocol cover majority of experiments

Step	Time	Temp	Additional Comment
A:Initial activation step	10 min	95°C	Enzyme activation
B:Amplification step			
Denaturation	15 s	95°C	
Annealing /Extension*	30-40	**60°C**	**Data acquisition**
Cycle number depends on the amount of template DNA	40-45		Cycle number
Melt curve Analysis **		**65 - 95°C**	

- In the case with low expression or peltier based real time, 3 step experiment also must be considered. In this case put **Data acquisition** step at **the end of extension.**

** For melt curve profile refer to instrument instruction.

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