

Product Information

MystiCq™ Universal PCR Primer

Catalog Number **MIRUP**

Storage Temperature -20 °C

Product Description

The MystiCq Universal PCR Primer has been designed and validated to work specifically with MystiCq microRNA qPCR Assay Primers and MystiCq microRNA SYBR® Green qPCR ReadyMix™ using first-strand cDNA produced with the MystiCq microRNA cDNA Synthesis Mix.

The MystiCq Universal PCR Primer targets a unique sequence at the 5'-end of the oligo-dT adapter primer used for cDNA synthesis as part of the MystiCq microRNA cDNA Synthesis Mix. microRNAs are not polyadenylated in nature. With the MystiCq microRNA cDNA Synthesis Mix, microRNAs are first polyadenylated in a poly(A) polymerase reaction (**Step 1**). ReadyScript™ Reverse Transcriptase and other necessary reagents for cDNA synthesis are subsequently added to convert the poly(A) tailed microRNAs into cDNA using an oligo-dT adapter primer (**Step 2**). The unique sequence at the 5' end of the adapter primer allows for amplification of microRNA cDNAs in real-time SYBR Green RT-qPCR reactions using the MystiCq Universal PCR Primer and individual MystiCq microRNA qPCR Assay Primers (**Step 3**). The pre-designed and validated MystiCq microRNA qPCR Assay Primers provide maximum sensitivity and specificity in RT-qPCR amplification and quantification of microRNAs.

Reagent

MystiCq Universal PCR Primer MIRUP-500RXN

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

MystiCq Universal PCR Primer is stable for 1 year when stored in a constant temperature freezer at -20 °C.

Procedure – Real Time SYBR Green RT-qPCR Amplification of MicroRNAs

Real-time SYBR Green RT-qPCR is performed using 200 nM of each MystiCq microRNA qPCR Assay Primer and MystiCq Universal PCR Primer along with the appropriate MystiCq microRNA SYBR Green qPCR ReadyMix product depending on the instrument platform being used. Please refer to the instrument reference table to select the proper formula for your instrument.

For each RT-qPCR reaction add the following components:

Reagent	Volume
MystiCq microRNA SYBR Green qPCR ReadyMix (2X)	25 µL
MystiCq microRNA qPCR Assay Primer (10 µM)	1.0 µL
MystiCq Universal PCR Primer (10 µM)	1.0 µL
Micro RNA cDNA	up to 23 µL
Nuclease-free water	variable
Total Volume	50 µL

The amount of microRNA cDNA can be adjusted depending on the expression level of the microRNA. As a starting point use about 1 ng of total RNA equivalent per RT-qPCR reaction. For microRNAs expressed at low levels you may use 10 ng of total RNA equivalent per RT-qPCR reaction. For most applications 20 to 25 µL RT-qPCR reaction volumes are suitable but reaction volumes can be scaled as needed.

2-Step Cycling Procedure (recommended)

Pre-incubation / activation	95 °C for 2 minutes
PCR (40 cycles)	
Denature	95 °C for 5 seconds
Anneal	60 °C for 30 seconds (collect fluorescence data)

3-Step Cycling Procedure (optional)

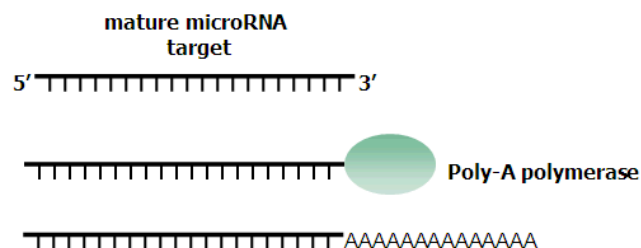
Pre-incubation / activation	95 °C for 2 minutes
PCR (40 cycles)	
Denature	95 °C for 5 seconds
Anneal	60 °C for 15 seconds
Extend	70 °C for 15 seconds (collect fluorescence data)

Use of a slightly higher annealing temperature (63 °C) in the 2-step Cycling Procedure may improve the specificity of some assays. Melt curve analysis is optional. Most microRNA RT-qPCR reactions will produce a single, slightly broader first-derivative melt peak compared to reactions using two gene-specific primers due to slight heterogeneity in the poly(A) tail length.

MicroRNA analysis using the MystiCq microRNA Quantification System.

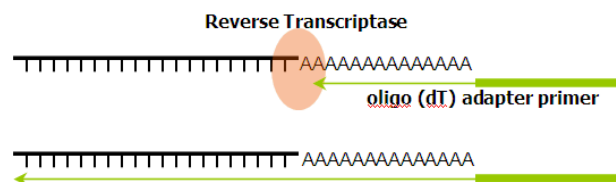
With the MystiCq microRNA cDNA Synthesis Mix, microRNAs are first polyadenylated in a poly(A) polymerase reaction (**Step 1**). ReadyScript Reverse Transcriptase and other necessary reagents for cDNA synthesis are subsequently added to convert the poly(A) tailed microRNAs into first-strand cDNA using an oligo-dT adapter primer (**Step 2**). The unique sequence at the 5' end of the adapter primer allows amplification of microRNA cDNAs in real-time SYBR Green RT-qPCR reactions using the MystiCq microRNA qPCR Universal Primer and individual MystiCq microRNA assays (**Step 3**).

Step 1. Polyadenylation of microRNAs



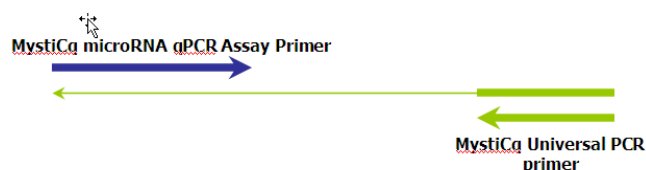
MystiCq microRNA cDNA Synthesis Mix (Catalog number MIRRT)

Step 2. First-strand cDNA synthesis



MystiCq microRNA cDNA Synthesis Mix (Catalog number MIRRT)

Step 3. Real-Time SYBR Green RT-qPCR amplification of microRNAs



MystiCq microRNA assays
MystiCq microRNA SYBR Green qPCR ReadyMix

Real-time PCR Instrument Name	MystiCq microRNA SYBR® Green qPCR ReadyMix	MystiCq microRNA SYBR Green qPCR ReadyMix Low ROX™	MystiCq microRNA SYBR Green qPCR ReadyMix with ROX	MystiCq microRNA SYBR Green qPCR ReadyMix iQ
	MIRRM00	MIRRM01	MIRRM02	MIRRM03
Applied Biosystems 5700			•	
Applied Biosystems 7000			•	
Applied Biosystems 7300			•	
Applied Biosystems 7500		•		
Applied Biosystems 7500 Fast		•		
Biosystems 7700Applied			•	
Applied Biosystems 7900			•	
Applied Biosystems 7900 HT Fast			•	
Applied Biosystems 7900HT			•	
Applied Biosystems StepOnePlus™			•	
Applied Biosystems StepOne™			•	
Applied Biosystems ViiA 7		•		
Bio-Rad CFX384™	•			
Bio-Rad CFX96™	•			
Bio-Rad iCycler iQ™				•
Bio-Rad iQ™5				•
Bio-Rad MiniOpticon™	•			
Bio-Rad MyiQ™				•
Bio-Rad/MJ Chromo4™	•			
Bio-Rad/MJ Opticon 2	•			
Bio-Rad/MJ Opticon®	•			
Cepheid SmartCycler®	•			
Eppendorf Mastercycler® ep realplex	•			
Eppendorf Mastercycler ep realplex2	•			
Illumina Eco qPCR	•			
Qiagen/Corbett Rotor-Gene® 3000	•			
Qiagen/Corbett Rotor-Gene 6000	•			
Qiagen/Corbett Rotor-Gene Q	•			
Roche LightCycler™ 480	•			
Stratagene Mx3000P®		•		
Stratagene Mx3005P™		•		
Stratagene Mx4000™		•		

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SC,PHC 05/12-1