3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

MystiCq™ microRNA cDNA Synthesis Mix
Catalog Number **MIRRT**
Storage Temperature –20 °C

Product Description

The MystiCq microRNA cDNA Synthesis Mix has been designed to easily convert microRNAs into cDNA templates for qPCR starting from total RNA or RNA preparations pre-enriched for microRNAs. However, recent studies have shown that Total RNA preparations and pre-enrichment for microRNAs is no longer necessary.

MicroRNAs are not naturally polyadenylated. With the MystiCq microRNA cDNA Synthesis Kit, microRNAs are polyadenylated through a poly(A) polymerase reaction. Then, 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. The adapter primer incorporates a unique sequence at its 5' end which allows for amplification of cDNAs in real-time RT-qPCR reactions.

The kit includes a Human Positive Control Primer that can be used to quantify a target gene that is ubiquitously expressed in most human tissues, the small nucleolar RNA SNORD44. There is sufficient Poly(A) Tailing Buffer and microRNA cDNA Reaction Mix to accommodate the use of no poly(A) polymerase and no reverse transcriptase control reactions.

Individual microRNAs are quantified in real-time SYBR® Green RT-qPCR reactions with the specific MystiCq microRNA qPCR Assay Primer and the MystiCq Universal PCR Primer (which binds specifically to the unique sequence incorporated into the cDNA by the oligo-dT adapter primer during the RT reaction). The pre-designed and validated MystiCq microRNA Assays provide maximum sensitivity and specificity in RT-qPCR amplification and quantification of microRNAs.

Reagents Provided

Description	Catalog No.	25 RXN	100 RXN
Poly (A) Tailing Buffer (5X)	MIRRT01	60 µL	240 µL
Human Positive Control Primer	MIRRT02	25 µL	100 µL
Nuclease-free Water	MIRRT03	1500 µL	1500 µL
MystiCq Universal PCR Primer	MIRRT04	125 µL	500 µL
Poly (A) Polymerase	MIRRT05	25 µL	100 µL
MystiCq microRNA cDNA Reaction Mix	MIRRT06	270 µL	1080 µL
ReadyScript Reverse Transcriptase	MIRRT07	25 µL	100 µL

Reagents Required, but not Provided

Description	Catalog No.	Vendor
MystiCq Universal PCR Primer (as necessary)	MIRUP-500RXN	Sigma-Aldrich
MystiCq microRNA qPCR Assay Primer and/or Control primer	various	Sigma-Aldrich
MystiCq microRNA SYBR Green qPCR ReadyMix™	Instrument dependent	Sigma-Aldrich

Precautions and Disclaimer

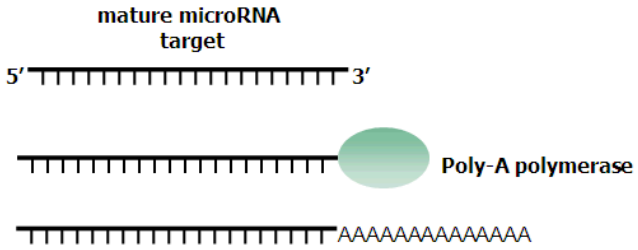
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

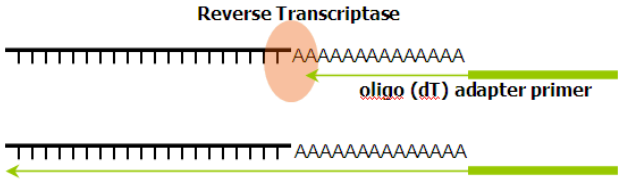
MystiCq microRNA cDNA Synthesis Mix is stable for 1 year when stored in a constant temperature freezer at -20 °C

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**).

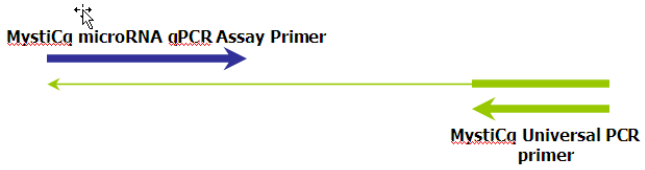
Step 1. Polyadenylation of microRNAs



Step 2. First-strand cDNA synthesis



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



Procedure

Poly(A) Tailing Reaction

1. After thawing components (except enzyme), place components on ice. Mix, and then briefly centrifuge to collect contents at the bottom of the tube.
2. Combine the following reagents in 0.2-mL micro-tubes or a 96-well plate sitting on ice.

Reagent	Volume
Poly (A) Tailing Buffer (5X)	2 µL
RNA (up to 1 µg total RNA or microRNA-enriched RNA)	Up to 7 µL
Nuclease-free water	variable
Poly (A) Polymerase	1 µL
Total Volume	10 µL

3. After sealing each reaction, vortex gently to mix contents. Centrifuge briefly to collect the components at the bottom of the reaction tube.
4. Incubate:
   60 minutes at 37 °C
   5 minutes at 70 °C
5. Briefly centrifuge to collect the contents. Keep on ice before cDNA synthesis. If using 100 ng or less of total RNA, the incubation at 37 °C can be shortened to 20 minutes

First-strand cDNA Synthesis Reaction

Set up the cDNA Synthesis Reaction:

Reagent	Volume
Poly (A) Tailing Reaction (from final step above)	10 µL
MystiCq microRNA cDNA Reaction Mix	9.0 µL
ReadyScript Reverse Transcriptase	1.0 µL
Total Volume	20 µL

6. After sealing each reaction, vortex gently to mix contents. Centrifuge briefly to collect the components.
7. Incubate
   20 minutes at 42 °C*
   5 minutes at 85 °C

*The RT reaction can be run at 45 °C to reduce background without affecting the sensitivity.

The cDNA is ready for RT-qPCR. If needed, the microRNA cDNA product can be diluted with water or 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA (recommended for long-term storage). Diluted cDNA is stable for several months at 4 °C. The cDNA can be stored long term 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
microRNA cDNA (0.1 – 10 ng)	variable
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 up or down 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)

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

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.

Quality Control

Kit components are free of contaminating DNase and RNase. ReadyScript Reverse Transcriptase is functionally tested in reverse transcription quantitative PCR (RT-qPCR). First-strand synthesis is performed in triplicate on each dilution of a log-fold serial dilution of HeLa cell total RNA from 1 pg to 1 µg. One-tenth of each first-strand reaction is used for qPCR amplification. Kinetic analysis must demonstrate linear resolution over five orders of dynamic range ($r^2 > 0.995$) and a PCR efficiency > 90%.

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 s	•			
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™		•		

Limited Label Licenses

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,994,056 and 6,171,785. The purchase of this product includes a limited, nontransferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

This product is provided under an agreement between Molecular Probes, Inc. (a wholly owned subsidiary of Invitrogen Corporation) and Quanta Biosciences, Inc., and the manufacture, use, sale or import of this product is subject to one or more of U.S. Patent Nos. 5,436,134; 5,658,751 and corresponding international equivalents, owned by Molecular Probes. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer, where such research does not include testing, analysis or screening services for any third party in return for compensation on a per test basis. The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851

The purchase of this product includes a limited, non-transferable license for all fields other than human or veterinary in vitro diagnostics under specific claims of U.S. Patent Nos. 6,174,670, 6,569,627 and 5,871,908, owned by the University of Utah Research Foundation or Evotec Biosystems GmbH and licensed to Idaho Technology, Inc. and Roche Diagnostics GmbH, to use only the enclosed amount of product according to the specified protocols. No right is conveyed, expressly, by implication, or by estoppel, to use any instrument or system under any claim of U.S. Patent Nos. 6,174,670, 6,569,627 and 5,871,908, other than for the amount of product contained herein.

Applicable only to products containing Passive Reference The purchase of this product includes a limited, non-transferable right to use the purchased amount of the product to perform Applied Biosystems' patented Passive Reference Method for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. For information about these rights or on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

SYBR is a registered trademark of Molecular Probes, Inc

MystiCq is a trademark of Quanta Biosciences,

ReadyMix and ReadyScript are trademarks of Sigma-Aldrich Co. LLC.

LightCycler is a registered Trademark of Roche.

Applied Biosystems, StepOne, StepOnePlus, ViiA, and ROX are trademarks Life Technologies Corporation.

Stratagene, MX3000P, MX3005P and MX4000 are trademarks of Agilent Technologies, Inc.

Mastercycler is a trademark of Eppendorf.

Rotor-Gene is a registered trademark of Qiagen GmbH. SmartCycler is a trademark of Cepheid.

CFX96, CFX384, iCycler iQ, iQ5, MyiQ, Opticon, MiniOpticon and Chromo4 are trademarks of Bio-Rad Laboratories

SC,ER,PHC 06/12-1