



ID3EAL miRNA qPCR ASSAY

Principle, Workflow and
Protocol



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ID3EAL miRNA qPCR Assay Technology and Principle

Re-defining miRNA Quantification with Sensitivity, Specificity and Speed

Key Benefits

Increased Sensitivity

Optimized RT-qPCR primers and reagents to drive efficient target amplification from limiting amounts (≥ 1 pg) of input RNA sample.

Improved Specificity

No universal primers. Every assay utilizes three miRNA specific primers to discriminate single nucleotide differences.

Speedy Detection

RNA to Ct in less than 2 hours for faster turnaround and improved throughput.

Reliable Data

Assays optimized by MIRXES' proprietary algorithm and wet-lab validated with synthetic miRNA templates and RNA from biological samples.

Convenience

Complete kit to minimize set-up time.
Compatible with all major qPCR instruments.

Assay Principle

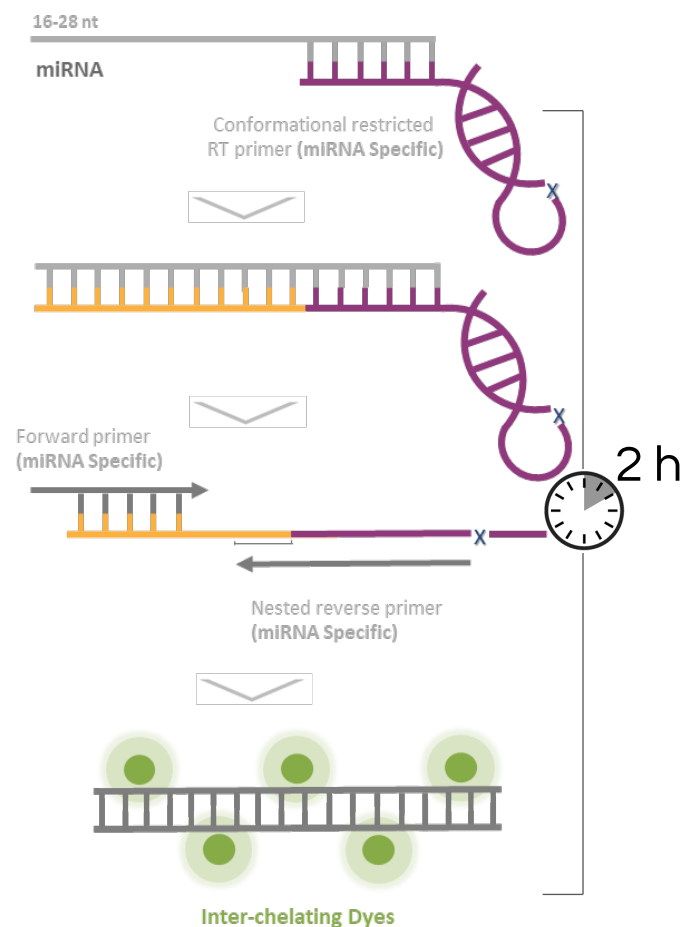


Figure 1. miRNA Assay Principle

Unique Features

Unique RT Primer: Conformational restricted miRNA specific RT primer efficiently hybridizes to mature but not precursor form of target miRNA.

Specific Real-Time PCR Primers: miRNA specific forward and reverse real-time PCR primers confer further specificity and enable robust amplification of amplicon.

Tailored RT-qPCR Reagents: Optimized RT and qPCR master mixes enhance signal to noise ratio.

ID3EAL miRNA qPCR Assay Kit Content

ID3EAL cDNA Synthesis System

Component	(20)	(60)	Storage Temperature
	1103101	1103103	
ID3EAL Reverse Transcriptase (20x)	1 x 20 µl	3 x 20 µl	-20 °C
ID3EAL miRNA RT Buffer (4x)	1 x 100 µl	3 x 100 µl	-20 °C

Component	(20)	(60)	(100)	Storage Temperature
	1103111	1103113	1103114	
ID3EAL Individual miRNA RT Primer 1-plex (20X)	1 x 20 µl	1 x 60 µl	1 x 100 µl	-20 °C

ID3EAL miRNA qPCR System

Component	(200)	(800)	(1200)	(2400)	Storage Temperature
	1104202	1104204	1104205	1104206	
ID3EAL miRNA qPCR Master Mix (2X)	2 x 1000 µl	8 x 1000 µl	12 x 1000 µl	24 x 1000 µl	-20 °C

Component	(200)	(800)	(1200)	(2400)	Storage Temperature
	1104212	1104214	1104215	1104216	
ID3EAL miRNA qPCR Master Mix – HR (2X)*	2 x 1000 µl	8 x 1000 µl	12 x 1000 µl	24 x 1000 µl	-20 °C

*For use in machines requiring Hi-ROX

Component	(100)	(500)	Storage Temperature
	1104101	1104103	
ID3EAL Individual miRNA qPCR Assay (2X)	1 x 200 µl	1 x 1,000 µl	-20 °C

Additional Equipment and Compatibility

Additional Equipment

As per good laboratory practices, always don the appropriate Personal Protective Equipment when handling chemicals or reagents. For additional information, consult the product Safety Data Sheets.

- Nuclease free labware (e.g. PCR tubes, pipette tips, microcentrifuge tubes, etc)
- Centrifuge suitable for PCR tubes/strips
- Cooling block or ice bucket suitable for PCR tubes/strips and reagents
- Vortex suitable for microcentrifuge tubes
- Heating blocks or a thermocycler capable of isothermal heating at 42°C and 70°C
- A compatible real-time PCR thermocycler

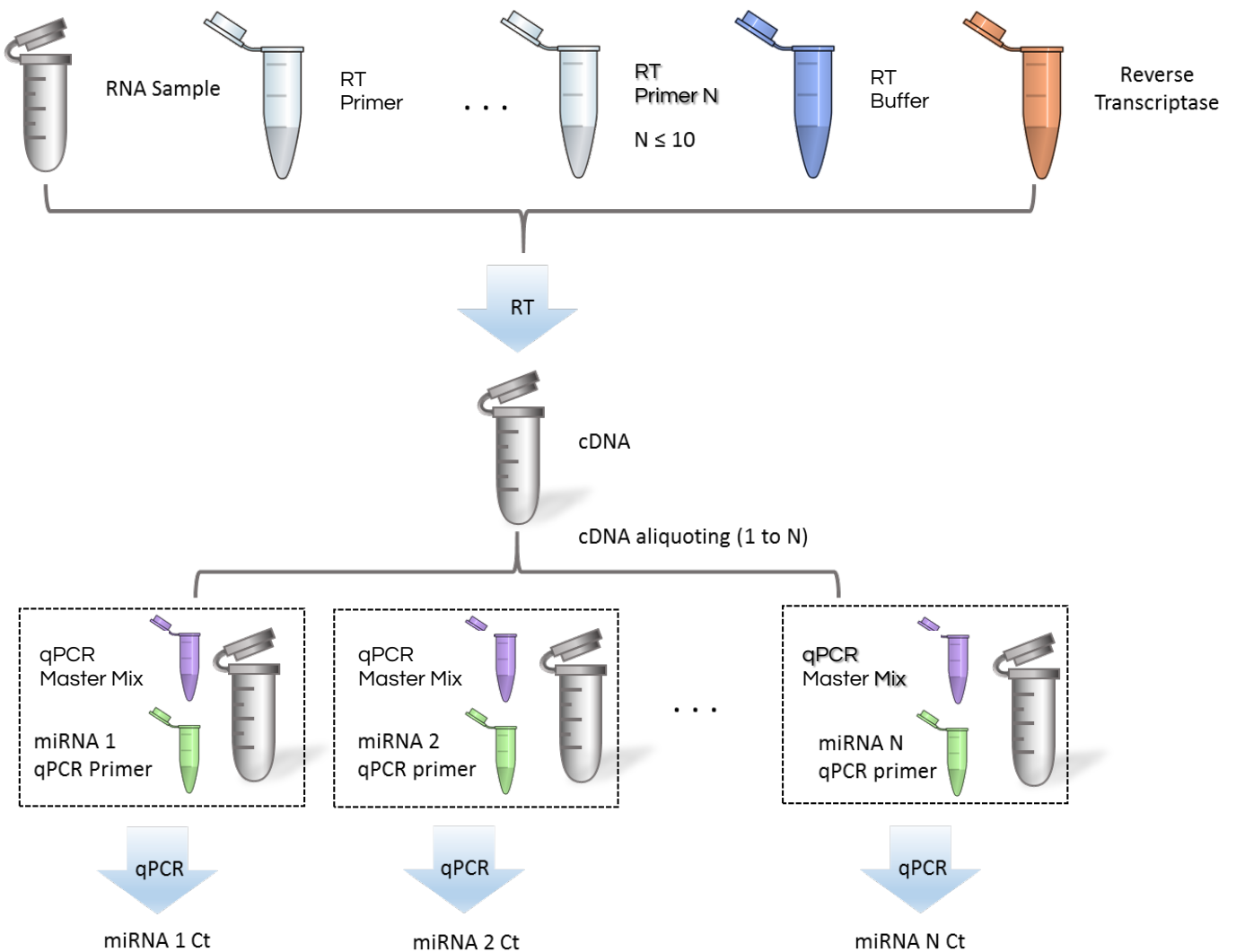
Wet Lab Validation with ID3EAL miRNA Reagents

MiRXES' assays have been optimized and wet-lab validated with ID3EAL miRNA cDNA Synthesis Kit and qPCR Master Mix. The assays have been validated using 20 µl reactions in 96-well plates and 10 µl reactions in 384-well plates. We do not recommend using lower reaction volumes as this may compromise fluorescence detection on some qPCR systems.

Workflow Option 1 : Multiplexed RT Assay

Multiplexed RT Assay Workflow

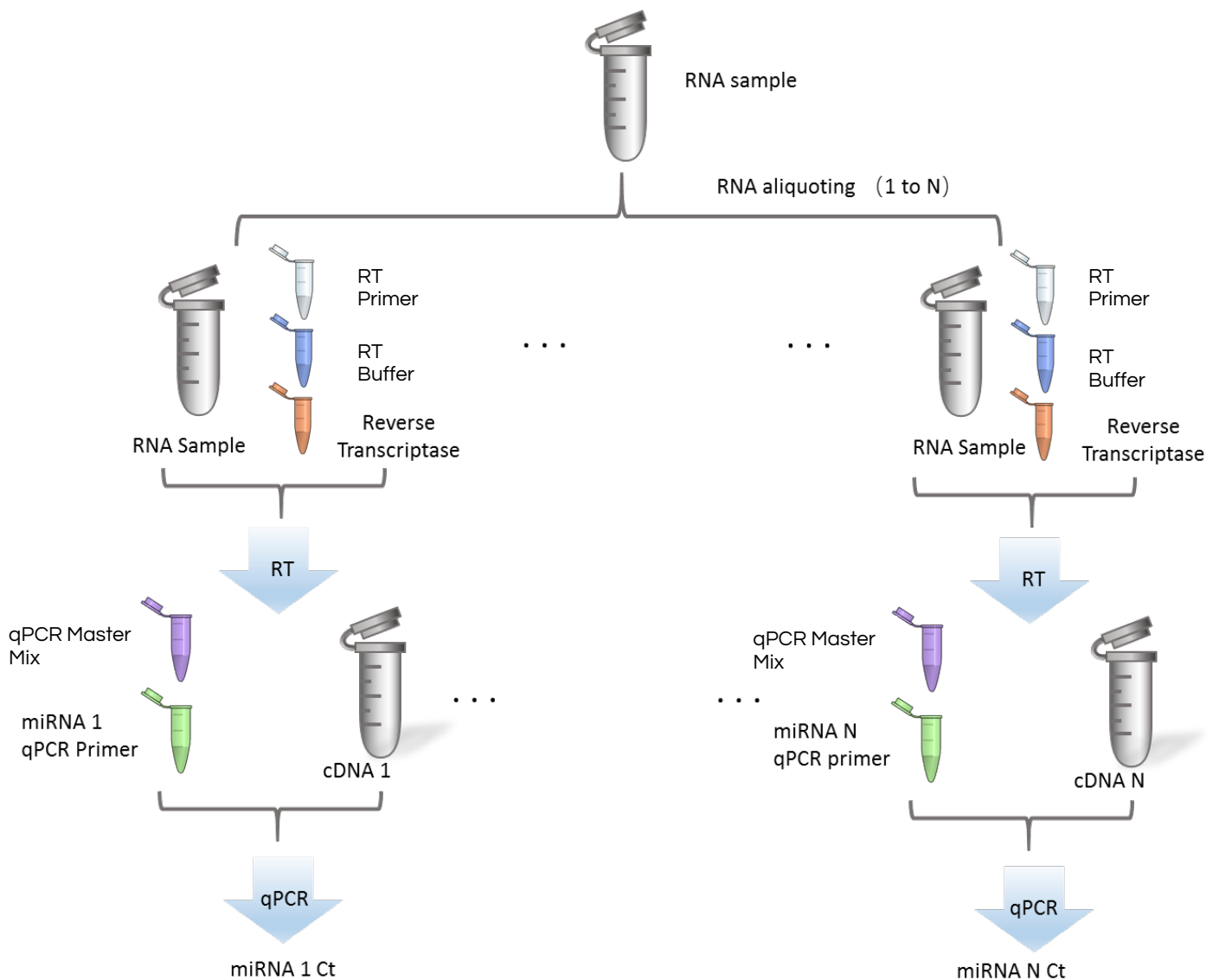
For testing miRNAs which are not highly homologues, reverse transcription can be multiplexed, follow the workflow shown below for easier handling. MiRXES™ recommends multiplex reactions of no more than 10 miRNAs at once.



Workflow Option 2: Singleplex RT

Singleplex RT

For testing miRNAs which are in the same miRNA family or closely resembling each other in sequences, MiRXES™ recommends single-plex reverse transcription. Aliquot RNA first and follow single assay protocol from **Step 1** to **Step 11**. The workflow shown below promises best specificity.



Reverse Transcription Protocol

Important: Keep all reagents on ice (or at 4°C) at all times during set up.

Stage I: Reverse Transcription

- Step 1:** Gently thaw **template RNA** on ice, use up to 1 µg per RT reaction.
- Step 2:** Thaw **ID3EAL miRNA RT Buffer (4x)** and **ID3EAL RT Primer 1-plexes (20x)**. Mix by vortexing and spin down by centrifugation. If necessary, incubate **ID3EAL miRNA RT Buffer (4x)** at 37°C and vortex to dissolve any precipitate.
- Step 3:** Assemble RT reaction according to Table 1. Reverse Transcriptase should be kept at -20°C and added to the master mix last.

Table 1 – Reverse transcription reaction setup (per reaction)

Reagent	Volume
Template RNA (up to 1 ug)	X µl
(Optional) ID3EAL RT Spike-In	1 µl
ID3EAL RT Buffer(4x)	5 µl
ID3EAL RT Primer 1-plex 1 (20x)	1 µl
ID3EAL RT Primer 1-plex N (20x) (N ≤ 10)	1 µl
ID3EAL Reverse Transcriptase (20x)	1 µl
Nuclease free water	To 20 µl
Total volume	20 µl

- Step 4:** Mix assembled reagents thoroughly and spin briefly.
- Step 5:** Incubate reaction at **42°C for 30 min** followed by heat-inactivation at **95°C for 5 min**

PAUSE POINT: Undiluted cDNA can be stored at -20°C for up to 4 weeks. Avoid repeated freeze-thaw cycles.

Real-time qPCR Protocol

Important: Keep all reagents on ice (or at 4°C) at all times during set up.

Stage II: Real-time qPCR amplification and detection

Step 6: Gently thaw cDNA, ID3EAL miRNA qPCR Master Mix (2x) and ID3EAL miRNA qPCR assays (10x) on ice. Mix by vortexing and spin down by centrifugation.

Step 7: Dilute cDNA from Step 5 by 1:10 in nuclease free water. Pipette diluted cDNA template to each PCR reaction well as indicated in Table 2.

Important: Keep PCR plate cool on cold block before loading qPCR master mix!

Step 8: Assemble qPCR reaction according to Table 2.

Table 2 – qPCR Reaction Setup (per reaction)

Reagent	Volume / μL (96 well)	Volume / μL (384 well)
ID3EAL qPCR Master Mix (2x)	10	5
Nuclease-free water	3	1
ID3EAL qPCR assays (10x)	2	1
Diluted cDNA	5	3
Total volume	20	10

Step 9: Centrifuge the PCR plate briefly (30 s at 200 g).

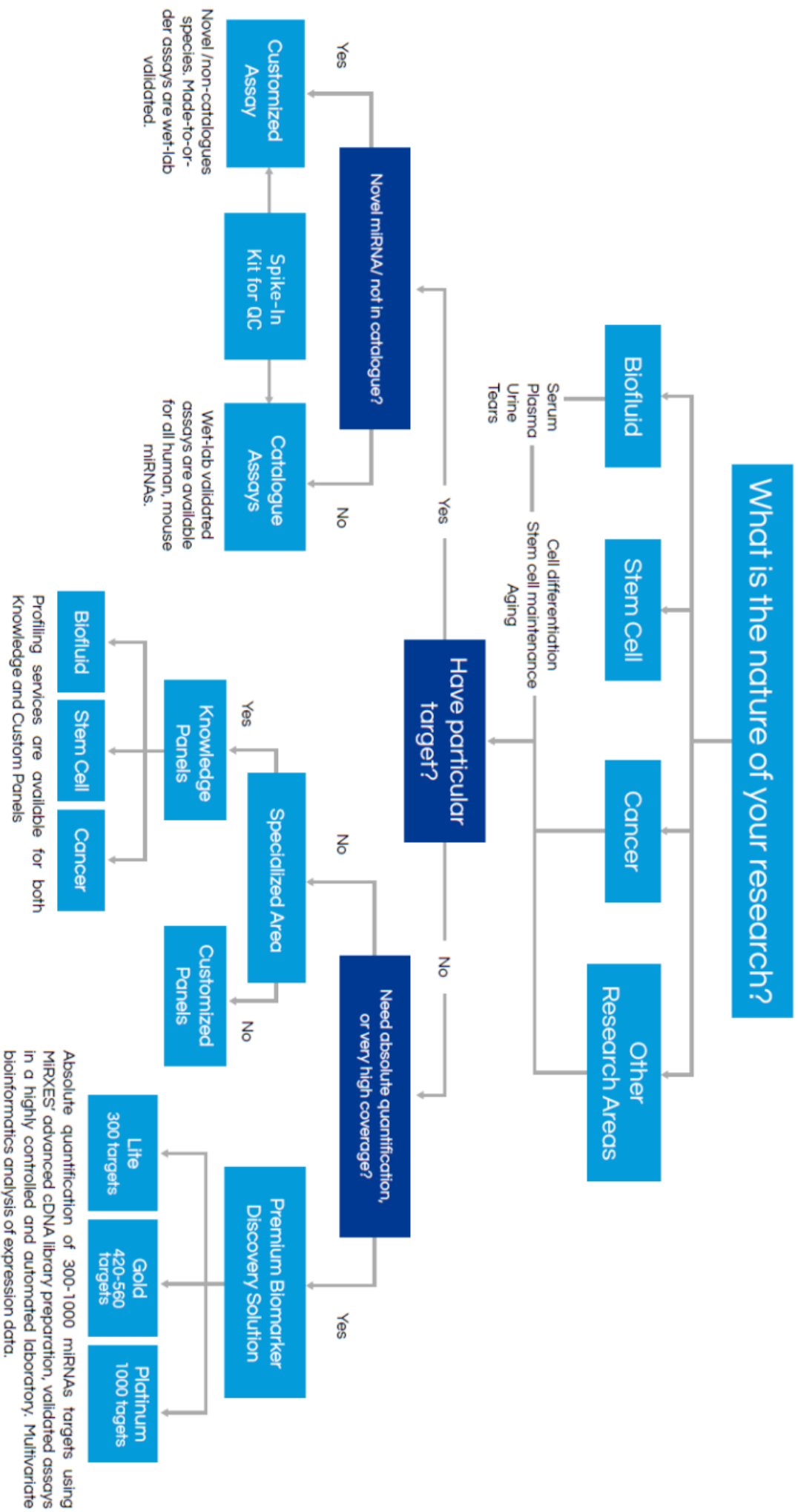
Step 10: Perform Real-time PCR amplification with the following cycling parameters.

Table 3 – real-time qPCR thermo-cycling protocol

Cycles	Temperature	Time	Notes
1x	95°C	10 min	Polymerase activation
	40°C	5 min	
40x	95°C	10 s	Denaturation
	60°C	30 s	Annealing/extension (acquire florescence reading at end of step)

Step 11: Data analysis.

How To Choose A Product/Service



ID3EAL miRNA qPCR ASSAY

Related Products

Product	SKU
ID3EAL Individual miRNA RT Primer 1-plex (20)	1103111
ID3EAL Individual miRNA RT Primer 1-plex (60)	1103113
ID3EAL Individual miRNA RT Primer 1-plex (100)	1103114
ID3EAL Individual miRNA qPCR Assay (100)	1104101
ID3EAL Individual miRNA qPCR Assay (500)	1104103
ID3EAL cDNA Synthesis System (20)	1103101
ID3EAL cDNA Synthesis System (60)	1103103
ID3EAL miRNA qPCR Master Mix (200)	1104202
ID3EAL miRNA qPCR Master Mix (800)	1104204
ID3EAL miRNA qPCR Master Mix (1200)	1104205
ID3EAL miRNA qPCR Master Mix (2400)	1104206
ID3EAL miRNA qPCR Master Mix - Hi-ROX (200)	1104212
ID3EAL miRNA qPCR Master Mix - Hi-ROX (800)	1104214
ID3EAL miRNA qPCR Master Mix - Hi-ROX (1200)	1104215
ID3EAL miRNA qPCR Master Mix - Hi-ROX (2400)	1104216
ID3EAL miRNA qPCR Starter Kit, 2 Targets (20)	1104301
ID3EAL miRNA qPCR Starter Kit, 2 Targets - Hi-ROX (20)	1104371
ID3EAL Complete Spike-in RNA Kit (50)	1102153
ID3EAL Complete Spike-in RNA Kit - Hi-ROX (50)	1102193
ID3EAL Spike-in control for Isolation (50)	1102122
ID3EAL Spike-in control for Reverse Transcription (50)	1102112
ID3EAL Multiplex miRNA RT Primers 96-plex (24)	1103151
ID3EAL Multiplex miRNA RT Primers 96-plex (72)	1103153
ID3EAL Customized Individual miRNA RT Primer 1-plex - For non-human and mouse miRNA (500)	1106104
ID3EAL Customized Individual miRNA qPCR Assay - For non-human and mouse miRNA (500)	1106173

Shipping and Storage

MiRXES™ miRNA Multi-Assay packs are shipped in both ice and dry ice. Upon receiving the pack, it should be stored in a constant temperature freezer at -20°C immediately. All components of the pack are promised to perform at an optimal level if proper handling and storage procedures are observed.

To further maintain the performance levels of the kit, it is highly recommended to store the miRNA qPCR Master Mix in aliquots in polypropylene tubes.

Product use limitations

This product is for research use only. 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. Not for diagnostic use.

Handling of this product should be done and observed with care and attention.

All users of this product are highly recommended to adhere to the various safety and handling guidelines that pertains to this particular product.

Product Warranty and Satisfaction Guarantee

MiRXES™ warrants that its products will conform to the standards stated in its product specification sheets in effect at the time of shipment. MiRXES™ will replace any product that does not conform to the specifications, free of charge. This warranty limits MiRXES™' liability to only the replacement of the product.

The technology employed in this product is covered by Patent No: 185776, SG; ZL 201180038333.8, CN; 5851496, JP. Patents pending in other nations.

The MiRXES™ terms and conditions can be obtained on request and also provided at the back of our invoices.

Any questions related to the product specifications and performances can be answered by contacting the MiRXES™ Technical Services, your distributor or by visiting ls.mirxes.com.

Safety Notes

At MiRXES™, we regard the safety of our customers and users of utmost importance. Appropriate personal protective equipment should be worn at all times when handling chemicals.

For more information on the product, please consult the relevant safety data sheets, which can be obtained from the distributor, or alternatively, contact the Technical Service Department.

In case of any accidents, contact the authorities that is relevant to your area or region.

MiRXES

To Know is to Act

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