ID3EAL Spike-in RNA Kit

Principle, Workflow and Protocol

MiRXES

To Know is to Act

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ID3EAL miRNA 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 (≥1pg) 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

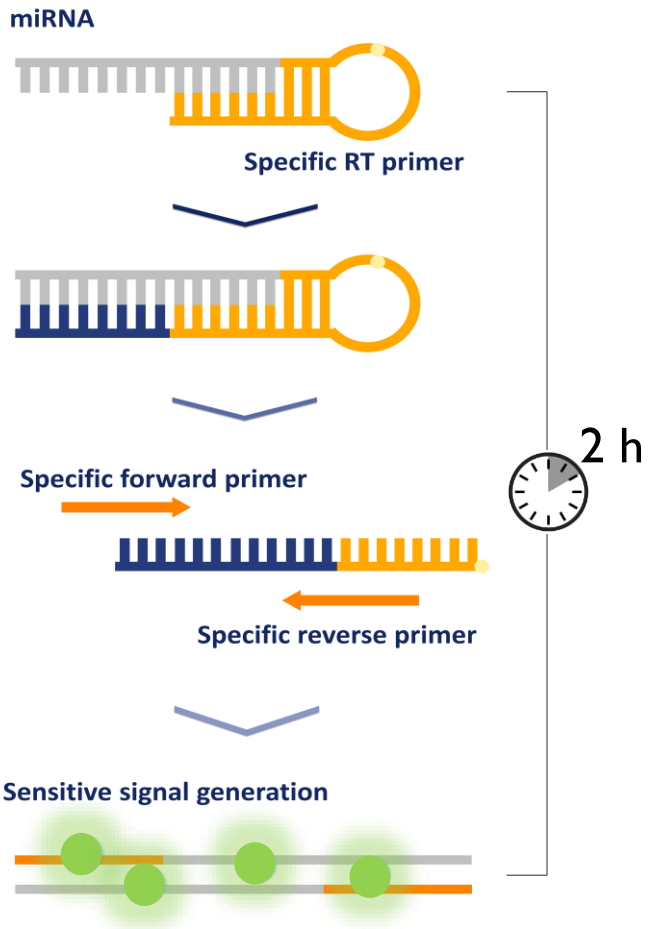


Figure 1. miRNA Assay Principle

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 Spike-In Kit Content

ID3EAL Spike-in RNA Kit

Component	SKU	Storage Temperature
ID3EAL Spike-in control for Isolation (50)	1102122	-20 °C -80 °C after reconstitution
ID3EAL cDNA Synthesis System (60) RT Primers incl below	1103103	-20 °C
ID3EAL miRNA qPCR Master Mix (2X) qPCR Primers incl below	1104202 / 1104212	-20 °C
ID3EAL Individual miRNA RT Primer 1-plex (20X) For Spike-in control for Isolation (Iso Spike-in 1)	1103113	-20 °C
ID3EAL Individual miRNA RT Primer 1-plex (20X) For Spike-in control for Isolation (Iso Spike-in 2)	1103113	-20 °C
ID3EAL Individual miRNA RT Primer 1-plex (20X) For Spike-in control for Reverse Transcription (RT Spike-in)	1103113	-20 °C
ID3EAL Individual miRNA qPCR Assay (100) For Spike-in control for Isolation (Iso Spike-in 1)	1104101	-20 °C
ID3EAL Individual miRNA qPCR Assay (100) For Spike-in control for Isolation (Iso Spike-in 2)	1104101	-20 °C
ID3EAL Individual miRNA qPCR Assay (100) For Spike-in control for Isolation (RT Spike-in)	1104101	-20 °C

Additional Reagents (not included)

Component	SKU	Storage Temperature
ID3EAL Spike-in control for Reverse Transcription (50)	1102112	-20 °C -80 °C after reconstitution

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	3 x 20 µl	5 x 20 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 100 µl	8 x 100 µl	12 x 100 µl	24 x 100 µl	-20 °C

Component	(200)	(800)	(1200)	(2400)	Storage Temperature
	1104212	1104214	1104215	1104216	
ID3EAL miRNA qPCR Master Mix – HR (2X)*	2 x 100 µl	8 x 100 µl	12 x 100 µl	24 x 100 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	5 x 200 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

Compatibility with Third Party cDNA Synthesis Kit

MiRXES' assays are compatible with most third party vendor's cDNA synthesis methods. User may use RT Primers as described in this manual. RT Primers can be added into third party's cDNA synthesis primer mix. Ensure that the final concentration are correct. However, qPCR must be performed with MiRXES' own reagent. User are advised to test the cDNA synthesis mix before actual experiment, MiRXES cannot guarantee the performance of assays if third party reagent is used.

Compatibility with ddPCR

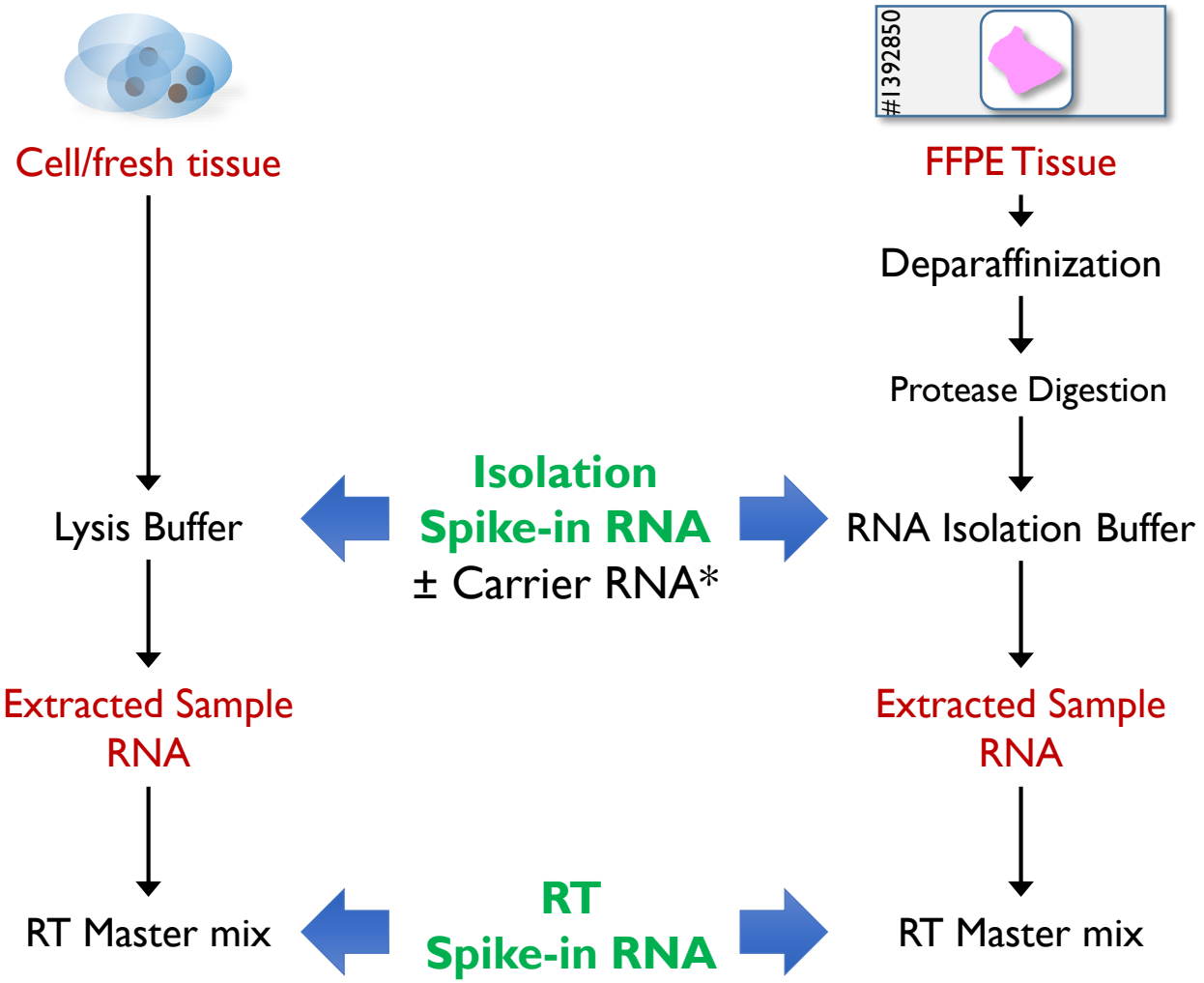
MiRXES' assays are compatible with ddPCR system. User are advised to optimize and test thermal cycling protocol if assays are to be run on ddPCR. Users may refer to manufacture's guideline on how to adapt qPCR protocol to ddPCR protocol. MiRXES cannot guarantee the performance of assays if third party regent is used.

Spike-in Protocol

There are two products in the ID3EAL miRNA Spike-ins :

1. **ID3EAL Spike-in control for Isolation (1102122)**
   Reconstitute with **275 µL** nuclease-free water before use (see Reconstitution Protocol). Add **5 µl** per sample of Isolation Spike-in to the sample lysis buffer or RNA isolation buffer before mixing with biological sample. A difference in the measured levels of Isolation Spike-Ins between samples indicates varying RNA isolation yield and/or RNA purity.
   Caution: RNA Spike-Ins should never be mixed with biological samples directly as it can be rapidly degraded by nucleases present in the samples.

2. **ID3EAL Spike-in control for Reverse Transcription (1102112)**
   Reconstitute with **55 µL** nuclease-free water before use (see Reconstitution Protocol). Add **1 µl** of the spike-in per reaction to RT master mix. Measured levels of RT Spike-In allow RT-qPCR normalization between RT reactions.



* Addition of carrier RNA, such as bacteriophage MS2 total RNA, is recommended to improve RNA isolation yield, when the biological sample is expected to yield only small amounts of RNA. Select carrier RNA that is guaranteed to be free from microRNAs.

Spike-In Reconstitution Protocol

ID3EAL Spike-Ins are shipped dry to improve their stability and performance. Follow the reconstitution protocol below to prepare them for use.

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

Step 1: Centrifuge the tube(s) at 1000 x g for 30 sec to ensure RNA pellet is at the bottom of the tube.

Step 2: Add nuclease free water to the tube as follows:

Product	Volume of nuclease free water
ID3EAL Spike-in control for Isolation (1102122)	275 µl
ID3EAL Spike-in control for Reverse Transcription (1102112)	55 µl

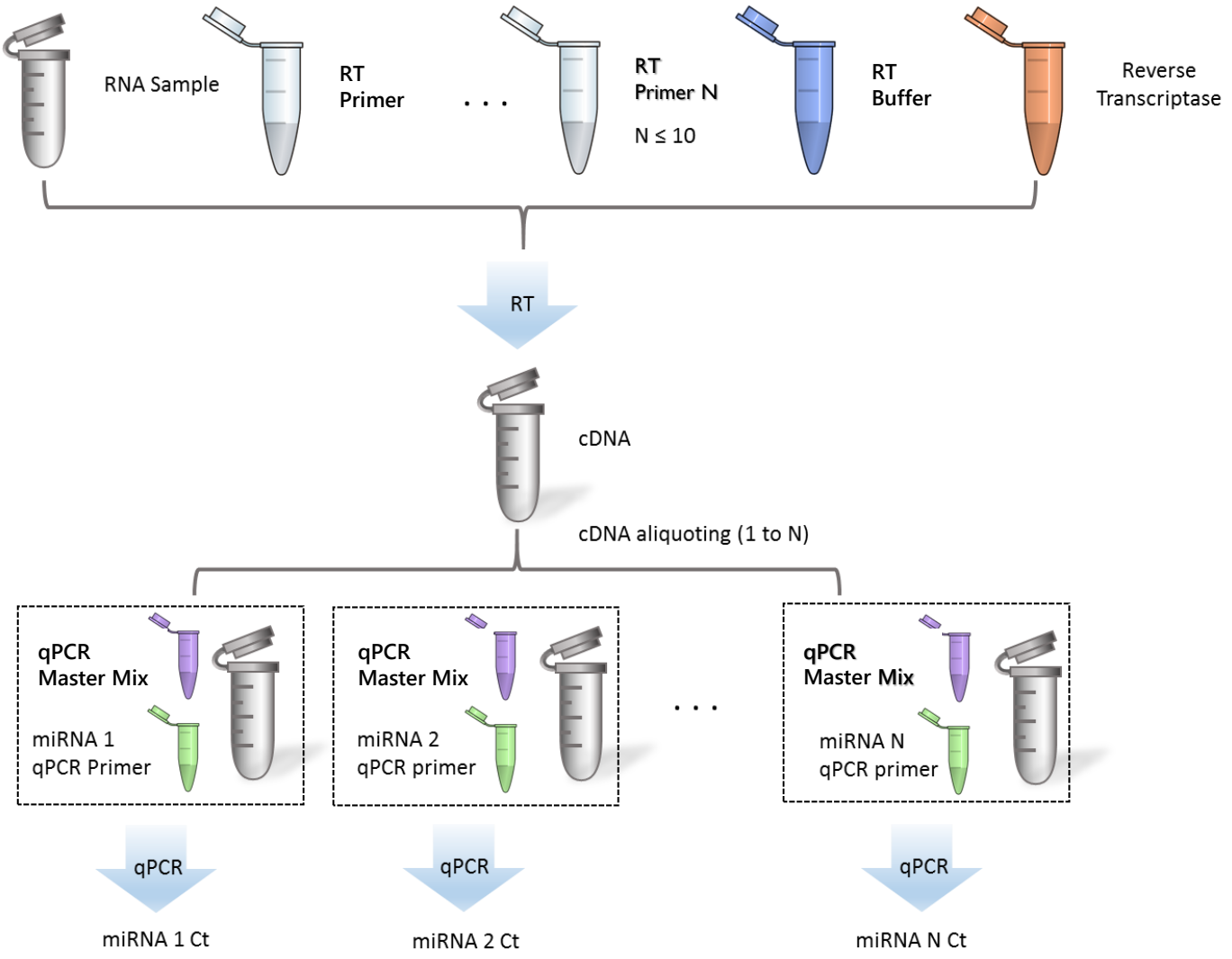
Step 3: Vortex well and spin down the solution.

Step 4: Store at -80 °C in aliquots or use immediately.

Workflow Option 1 : Multiplexed RT Assay

Multiplexed RT Assay Workflow

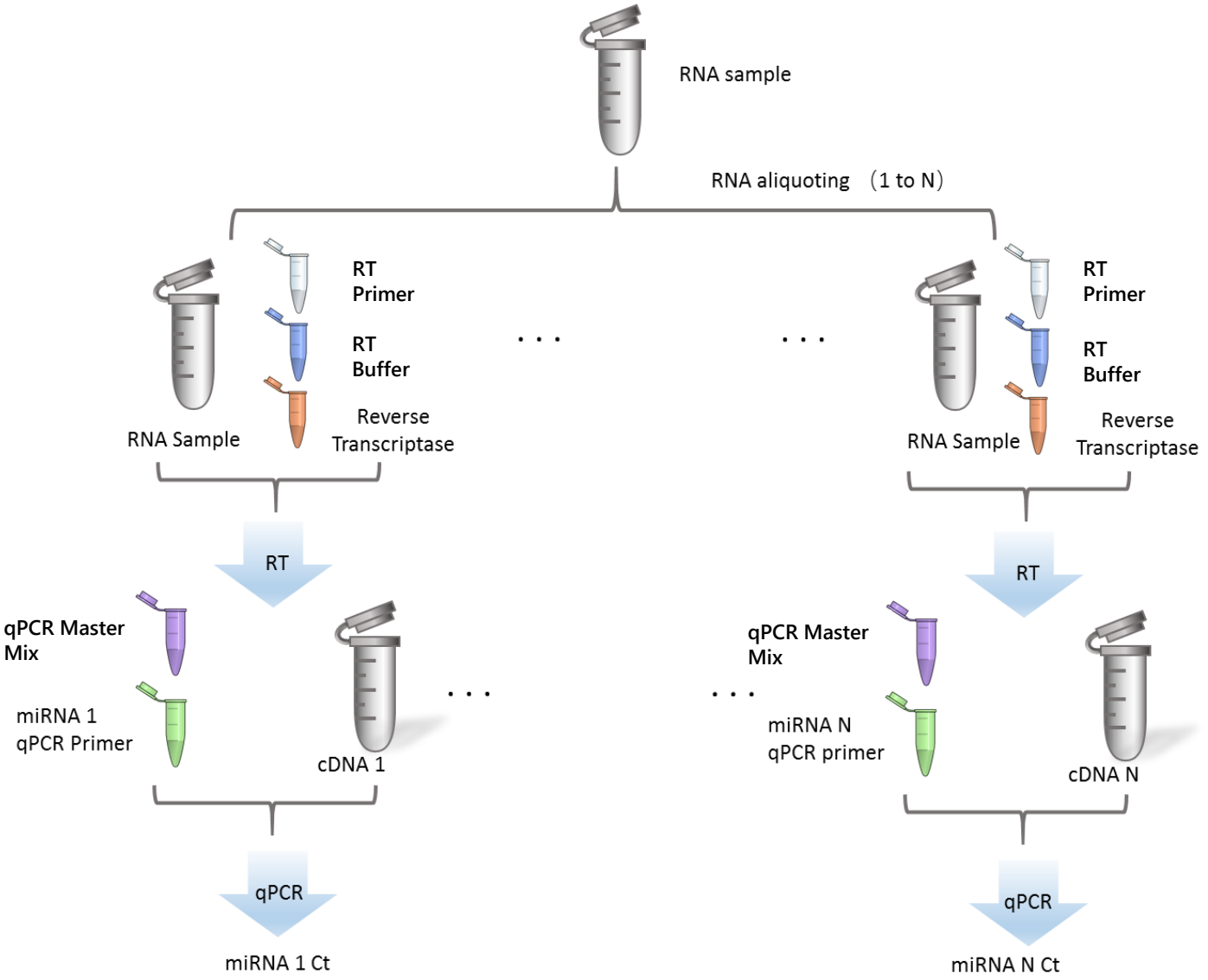
For testing miRNAs which are not highly homologues, reverse transcription can be multiplexed, follow the workflow shown below for easier handling. MiRXES™ recommends multiplex reactions of no more than 10 miRNAs at once.



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.



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
ID3EAL RT Spike-In RNA (if applicable)	1 µl
ID3EAL miRNA RT Buffer(2x)	10 µl
RT Primer 1-plex 1 (20x)	1 µl
RT Primer 1-plex N (20x) (N ≤ 10)	1 µl
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 -20ºC for up to 4 weeks. Avoid repeated freeze-thaw cycles.

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 Assay (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).

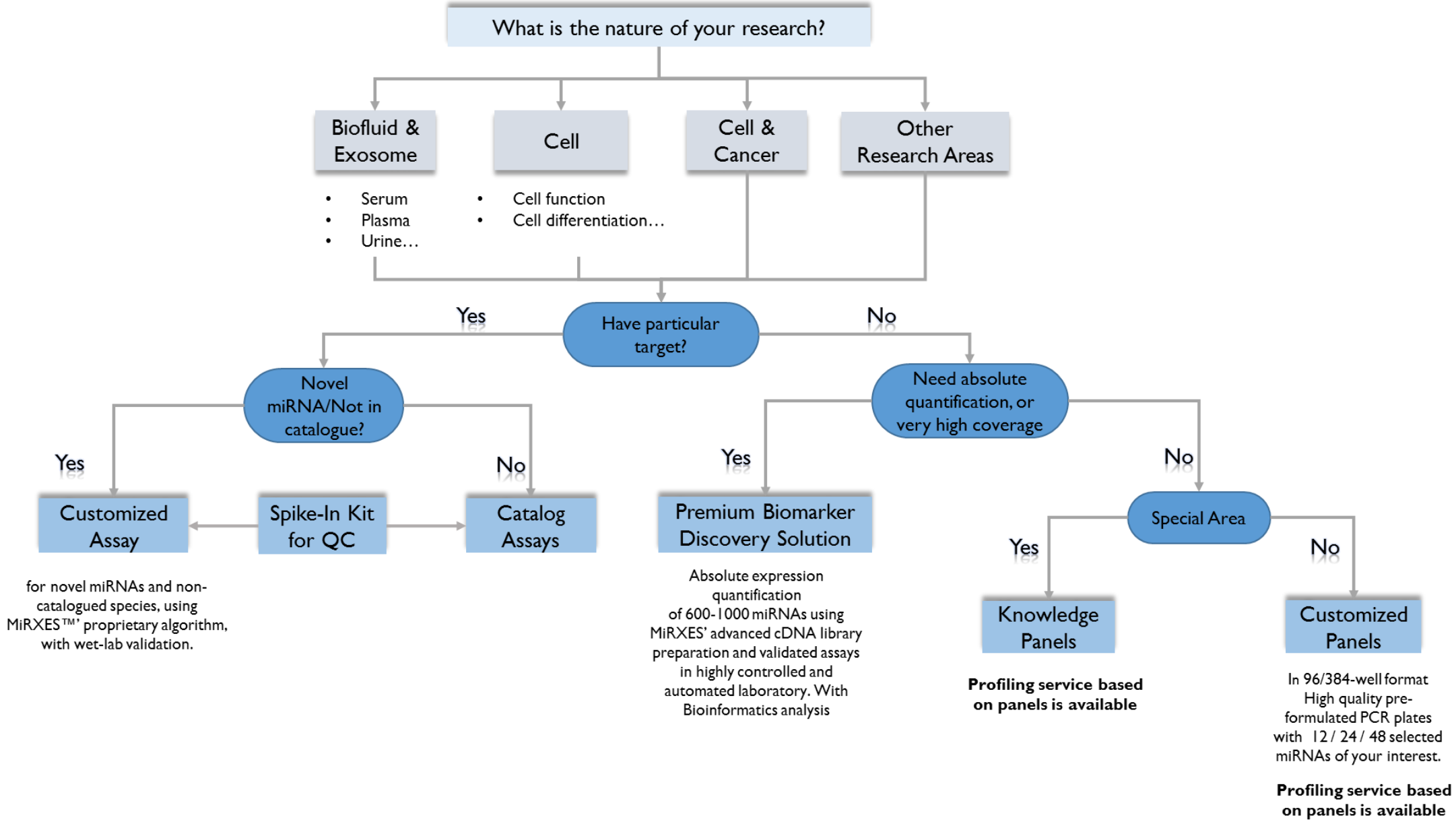
Step 10: Perform Real-time PCR amplification with the following cycling parameters.

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



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 unless otherwise noted. All components of the pack will perform at a 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 www.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

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