



IDEAL™

Spike-In RNA Kit

Individual Assays
Principle, Workflow and Protocol



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IDEAL 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 (≥ 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

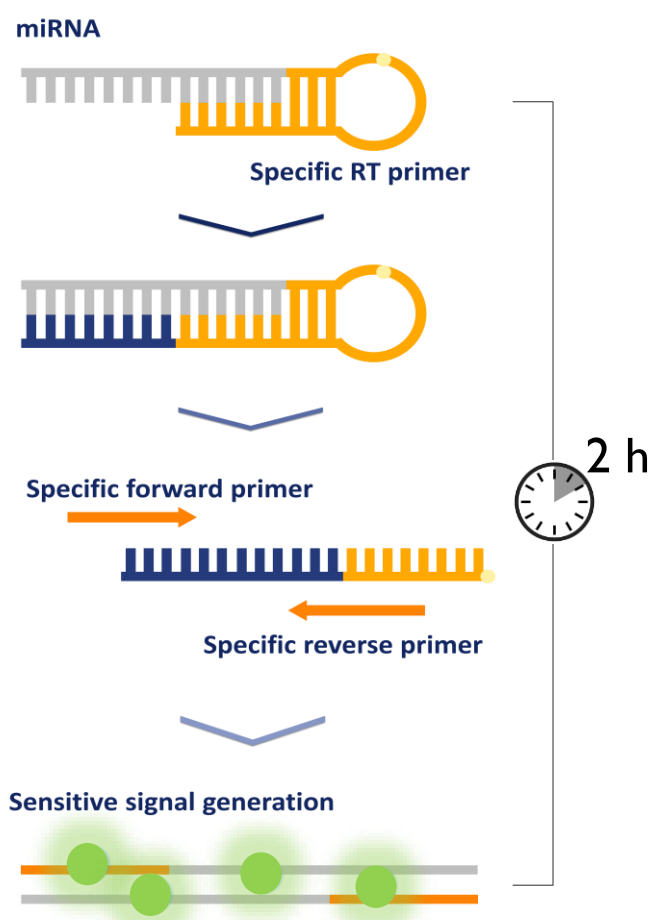


Figure 1. miRNA Assay Principle

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.

IDEAL™ Spike-In RNA Kit

Kit Content

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

KIT CONTENTS

Component	Kit
IDEAL Spike-In miRNA RT Primers (50x) <ul style="list-style-type: none"> RNA Spike-In 1 RNA Spike-In 2 	10 µl per assay, sufficient for 50 RT reactions (10 µl reactions)
IDEAL miRNA RT Buffer(2x)	250 µl, sufficient for 50 RT reactions (10 µl reactions)
Reverse Transcriptase (20x)	25 µl, sufficient for 50 RT reactions (10 µl reactions)
IDEAL miRNA qPCR Assay (10x) <ul style="list-style-type: none"> RNA Spike-In 1 RNA Spike-In 2 	200 µl per assay, sufficient for 100 qPCR reactions for each assay (20 µl reactions)
IDEAL miRNA qPCR Master Mix (2x)	1000 µl per assay, sufficient for 100 qPCR reactions for each assay (20 µl reactions)
RNA Spike-In <ul style="list-style-type: none"> RNA Spike-In 1 (2 x 10⁶ copies per µl) RNA Spike-In 2 (2 x 10⁸ copies per µl) 	250 µl, sufficient for 50 samples (5 µl per sample isolation or 1 µl per RT reaction)

KIT STORAGE

Component	Kit
IDEAL miRNA RT Primers (50x)	-20°C
IDEAL miRNA RT Buffer(2x)	-20°C
Reverse Transcriptase (20x)	-20°C
IDEAL miRNA qPCR Assay (10x)	-20°C
IDEAL miRNA qPCR Master Mix (2x)	-20°C

IDEAL™ Spike-In RNA Kit

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 oligos as described in this manual. RT Oligos 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 reagent is used.

IDEAL™ Spike-In RNA Kit Workflow

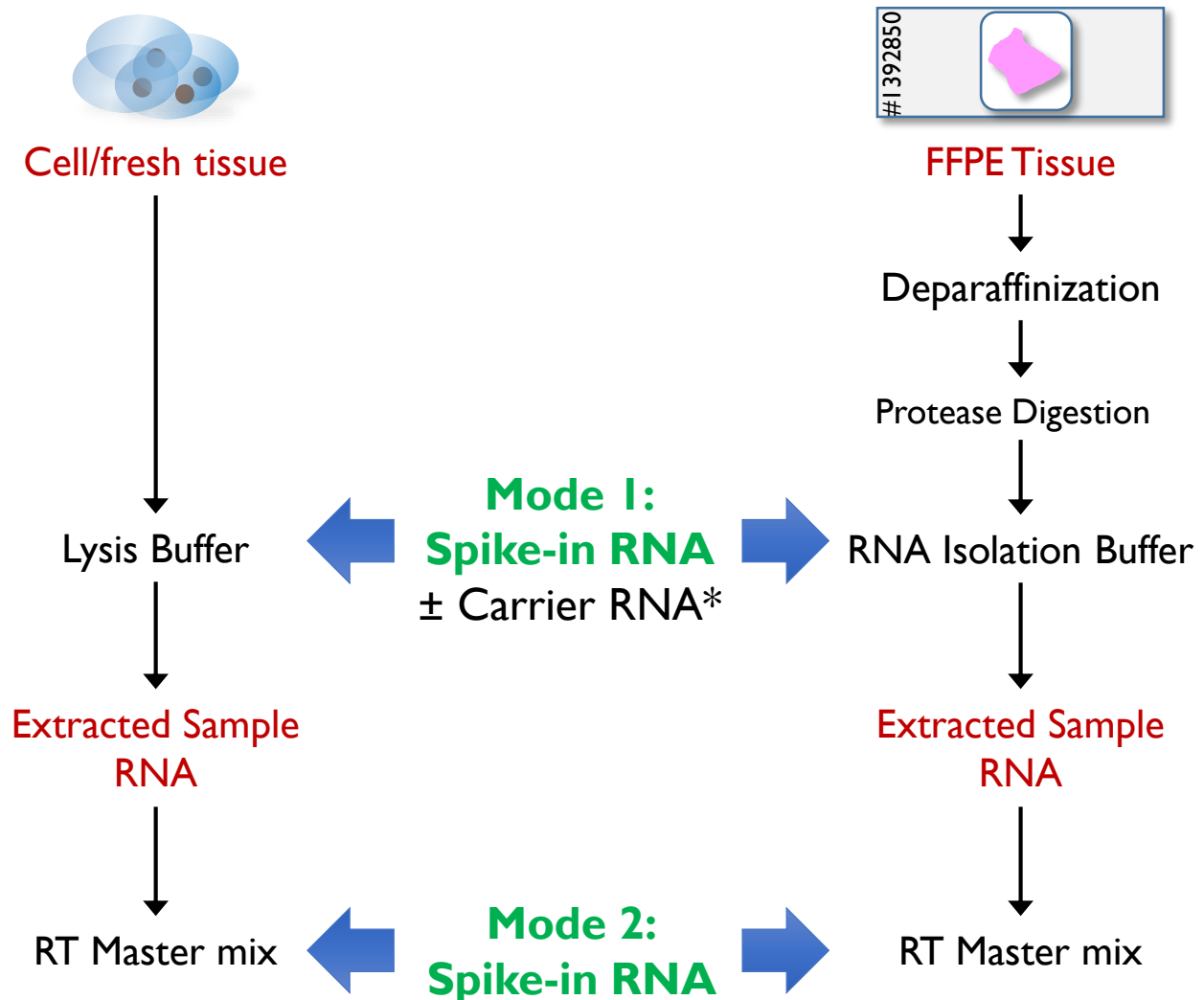
The miRNA spike-ins can be used in **two modes**:

1. **RNA isolation control.** Add **5 μ l** of the spike-in per sample to sample lysis buffer or RNA isolation buffer before mixing with biological sample. A difference in the measured levels of miRNA spike-ins between samples indicates varying RNA isolation yield and/or RNA purity.

Caution: miRNA spike-in mix should never be mixed with biological samples directly as it can be rapidly degraded by nucleases present in the samples.

2. **Reverse transcription control.** Add **1 μ l** of the spike-in per reaction to RT master mix. Measured levels of miRNA spike-ins allow normalization between RT reactions.

Note: It is not possible to use the same miRNA spike-ins for both modes in the same experiment.



* 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.

IDEAL™ Spike-In RNA Kit

miRNA Spike-In 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 100 ng of total RNA per 10 µl RT reaction.
- Step 2:** Gently thaw miRNA RT Buffer(2x) and RT Oligo (50x) on ice. Mix by vortexing and spin down by centrifugation. Incubate miRNA RT Buffer(2x) at 37°C and vortex to dissolve any precipitate.
- Step 3:** Assemble RT reaction according to Table I. Reverse Transcriptase should be kept at -20°C and added to the master mix last. ***Only add the RNA Spike-In if it has not already been added during the isolation step.**

Table I – Reverse Transcription Reaction Setup (per reaction)

Reagent	Volume
Template RNA (up to 100 ng)	X µl
Nuclease free water (*Optional : 1 µl of RNA Spike-In)	4.5-0.2N-X µl
miRNA RT Buffer(2x)	5 µl
RT Oligo 1 (50x)	0.2 µl
RT Oligo 2 (50x)	0.2 µl
...	0.2 µl
RT Oligo N (50x) (N ≤ 10)	0.2 µl
Reverse Transcriptase	0.5 µl
Total volume	10 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.

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miRNA Spike-In 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, miRNA qPCR Master Mix (2x) and miRNA qPCR assays (10x) on ice. Mix by vortexing and spin down by centrifugation.

Step 7: Dilute cDNA template 10 times in nuclease free water. Pipette 5 µl diluted cDNA template to each PCR reaction well.

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

Step 8: Assemble qPCR reaction according to Table 2. Dispense 15 µl of qPCR master mix per well

Table 2 – qPCR Reaction Setup (per reaction)

Reagent	Volume
miRNA qPCR Master Mix (2x)	10 µl
Nuclease-free water	3 µl
miRNA qPCR assays (10x)	2 µl
Diluted cDNA	5 µl
Total volume	20 µl

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

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

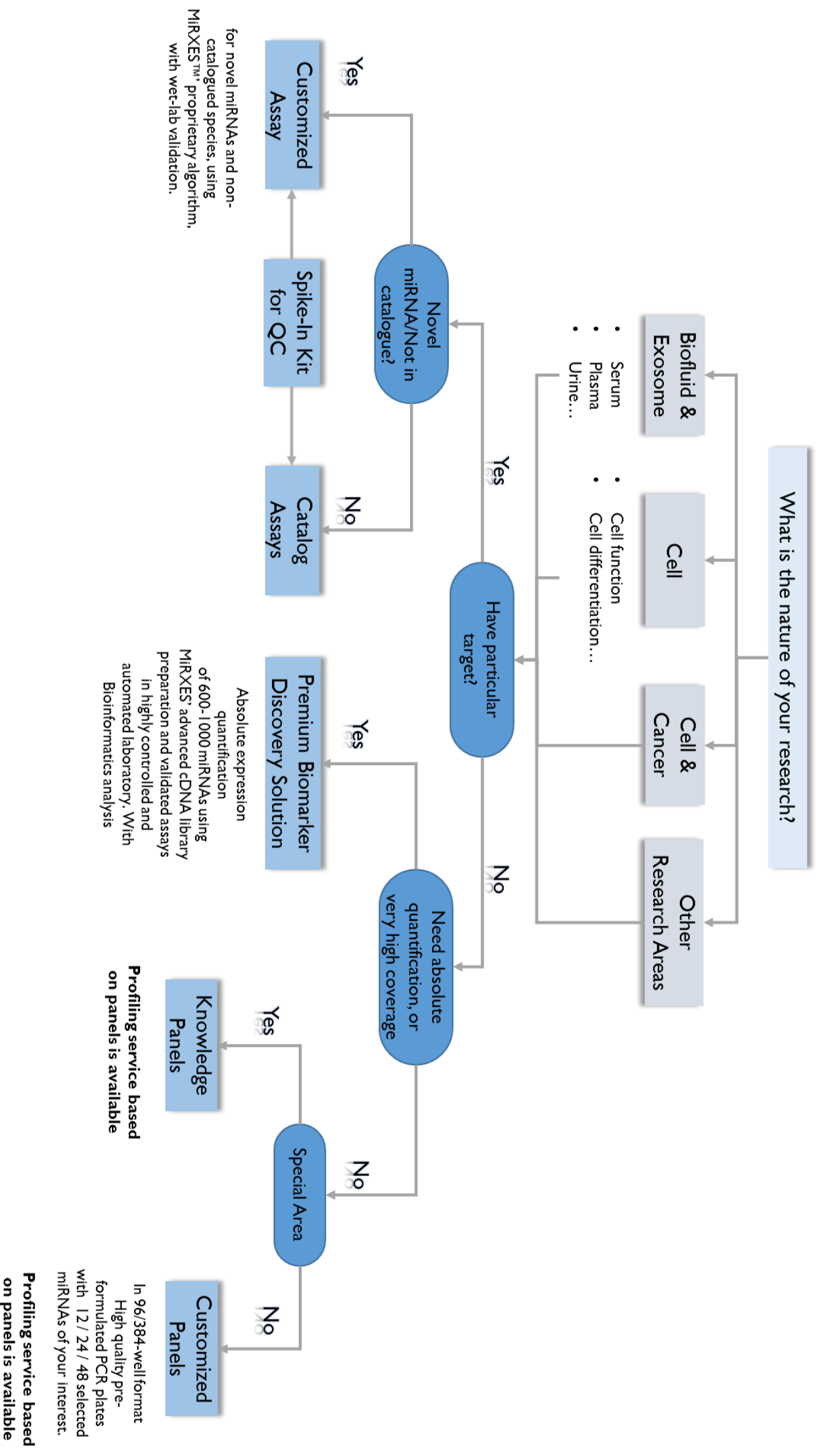
Table 3 – real-time qPCR thermo-cycling protocol

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 fluorescence reading at end of step)

Step 11: Data analysis.

RNA Spike-In	Expected C _t
RNA Spike-In 1	25
RNA Spike-In 2	20

How To Choose A Product/Service



IDEAL™ Spike-In RNA Kit

Related Products

Product	Cat No.	Description
IDEAL Individual miRNA qPCR Assay (2-miRNA pack)	MX003-2	50 x 10 µL RT reactions and 100 x 20 µL qPCR reactions (sufficient for 2 miRNAs, multiplexed RT)
IDEAL Individual miRNA qPCR Assay (4-miRNA pack)	MX003-4	50 x 10 µL RT reactions and 100 x 20 µL qPCR reactions (sufficient for 4 miRNAs, multiplexed RT)
IDEAL Individual miRNA qPCR Assay (10-miRNA pack)	MX003-10	50 x 10 µL RT reactions and 100 x 20 µL qPCR reactions (sufficient for 10 miRNAs, multiplexed RT)
IDEAL Individual miRNA qPCR Assay (qPCR Top-up)	MX003-T	50 x 20 µL qPCR reactions, per miRNA (inc qPCR assay and mastermix only)
IDEAL Spike-in RNA Kit	MC001	Set of 2 pre-mixed spike-in RNAs, sufficient to spike into 50 samples. With companion assays and reagents for 50 RT reactions and 100 qPCR reactions

Product	Cat No.	Description
IDEAL Biofluid miRNA Knowledge Panel (2-plate pack)	MP001-2	Measures 176 Biofluid miRNAs. Sufficient reagents (including RNA spike-in) for 2 samples.
IDEAL Biofluid miRNA Knowledge Panel (6-plate pack)	MP001-6	sufficient for 6 samples
IDEAL Biofluid miRNA Knowledge Panel (12-plate pack)	MP001-12	sufficient for 12 samples
IDEAL Biofluid miRNA Knowledge Panel (24-plate pack)	MP001-24	sufficient for 24 samples

Product	Cat No.	Description
IDEAL Stem Cell miRNA Knowledge Panel (2-plate pack)	MP002	Measures 176 Cell miRNAs. Sufficient reagents (including RNA spike-in) for 2 samples.
IDEAL Stem Cell miRNA Knowledge Panel (6-plate pack)	MP002-6	sufficient for 6 samples
IDEAL Stem Cell miRNA Knowledge Panel (12-plate pack)	MP002-12	sufficient for 12 samples
IDEAL Stem Cell miRNA Knowledge Panel (24-plate pack)	MP002-24	sufficient for 24 samples

Product	Cat No.	Description
IDEAL Cancer miRNA Knowledge Panel (2-plate pack)	MP003	Measures 352 Cancer miRNAs. Sufficient reagents (including RNA spike-in) for 2 samples.
IDEAL Cancer miRNA Knowledge Panel (6-plate pack)	MP003-6	sufficient for 6 samples
IDEAL Cancer miRNA Knowledge Panel (12-plate pack)	MP003-12	sufficient for 12 samples
IDEAL Cancer miRNA Knowledge Panel (24-plate pack)	MP003-24	sufficient for 24 samples

IDEAL™ Spike-In RNA Kit

Additional Information

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 pertain 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: I85776, 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.



IDEAL™ Spike-In RNA Kit

Additional Information

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

For Research Use Only. Not for use in diagnostic procedures.

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