

# MIRXES

## ID3EAL miRNA Starter qPCR Kit

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 ( $\geq 1$  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

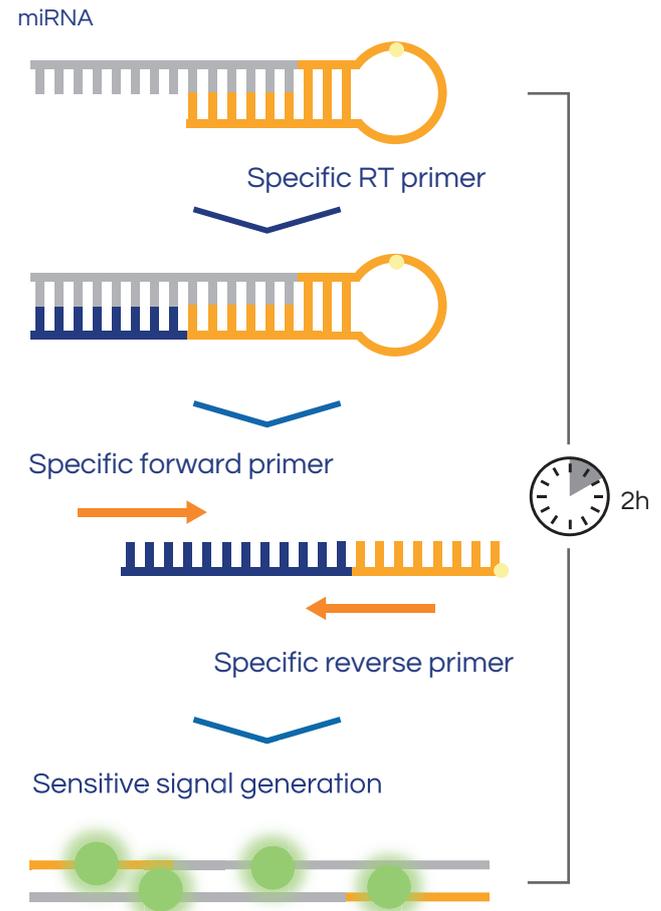


Figure 1. miRNA Assay Principle

### 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.

MiRXES ID3EAL miRNA qPCR Starter Kit provides the perfect solution for research groups generating initial microRNA data. It contains all reagents required to perform 20 cDNA reactions and 200 PCR reactions. The MiRXES ID3EAL miRNA qPCR Starter Kit allows users to pick two validated primer sets of choice\*.

MiRXES ID3EAL Individual miRNA qPCR Assays have been optimized to provide accurate, microRNA detection on most real-time PCR cyclers.

Wet-lab validated assays in accordance with the MIQE guidelines.

High-performance proprietary qPCR Master Mix specifically designed for the MiRXES ID3EAL microRNA PCR system.

MiRXES' patented miRNA RT-qPCR technology delivers results from RNA samples in just 2 hours.

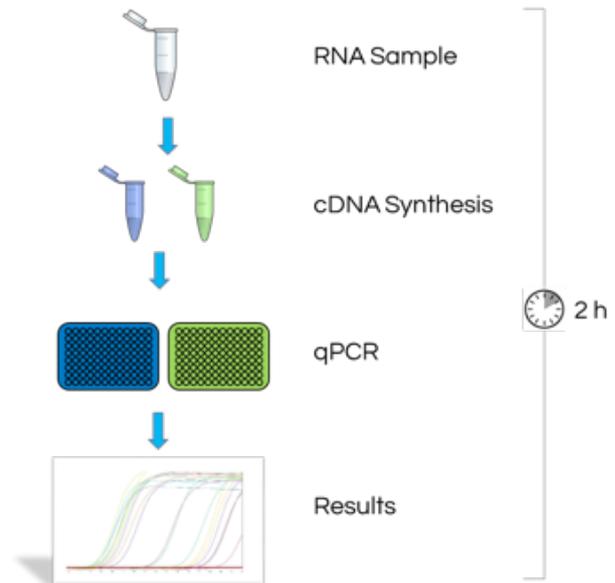


Figure 1. The figure shows various format of the ID3EAL Customized Knowledge Panel. 96-well format will be able to support 24-96 different microRNA targets. 384-well format will be able to support 48-384 different microRNA targets

## Unparalleled Specificity

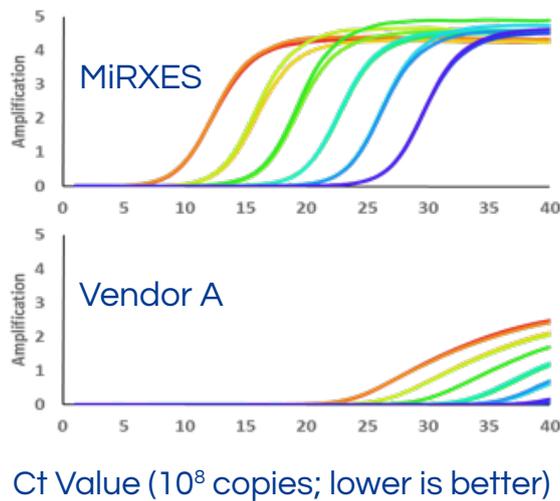
The combination of miRNA specific RT primer and nested qPCR primer pairs enables assays to efficiently discriminate highly homologous miRNA family members with single nucleotide difference.

Target	Assay relative detection								
	let-7a	let-7b	let-7c	let-7d	let-7e	let-7f	let-7g	let-7i	miR-98
UGAGGUAGUAGGUUUGUAUAGUU let-7a	100.00%	0.00%	0.10%	1.20%	0.00%	0.50%	0.00%	0.00%	0.00%
UGAGGUAGUAGGUUGUGUGUU let-7b	0.00%	100.00%	2.20%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
UGAGGUAGUAGGUUGUAUGUU let-7c	0.80%	1.60%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
AGAGGUAGUAGGUUGCAUAGUU let-7d	0.40%	0.00%	0.10%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%
UGAGGUAGGAGGUUGUAUAGUU let-7e	0.10%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%
UGAGGUAGUAGAUUGUAUAGUU let-7f	0.10%	0.00%	0.00%	0.00%	0.10%	100.00%	0.00%	0.00%	0.00%
UGAGGUAGUAGUUGUACAGUU let-7g	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%
UGAGGUAGUAGUUGUGCUGUU let-7i	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%
UGAGGUAGUAGUUGUAUUGUU miR-98	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%

Figure 2. Single Nucleotide Discrimination Test. Single base differences occur frequently, especially in microRNAs that are closely related. This is a common challenge in microRNA quantification. The above figure includes the RNA sequences of the let-7 microRNA family. Base changes are highlighted in orange. Synthetic cDNA was used as a template for each of the isoform. The percentage relative detection was calculated from the CT values collected from matching and mis-matched assays. Realtime PCR technology demonstrates significantly higher competency in discriminating the let-7 microRNA family members. Using MiRXES proprietary thermodynamics based algorithms, MiRXES ID3EAL microRNA PCR system was able to deliver efficient specificity down to a single base difference.

### Superior Sensitivity

Many microRNAs are expressed at relatively low levels and demonstrate subtle changes. MiRXES ID3EAL microRNA PCR system was designed to yield class leading sensitivity when compared to other microRNA detection systems. MiRXES ID3EAL microRNA PCR System excels in sensitivity, a broad dynamic range, and consistently high amplification efficiencies, especially in difficult samples such as plasma. MiRXES ID3EAL microRNA PCR system excels in sensitivity, a broad dynamic range, and consistently high amplification efficiencies.



miRNA	% AT	MiRXES	Vendor A	Vendor B	Vendor C
hsa-miR-27a-5p	45%	12.6	16.5	18.1	N/A
hsa-miR-500a-5p	52%	12.5	16.3	19.8	15.8
hsa-miR-30e-3p	55%	13.1	14.8	17.2	14.5
hsa-miR-215-5p	62%	12.8	16.0	19.5	15.6
hsa-miR-32-3p	73%	13.0	21.1	21.2	N/A

Figure 3. Amplification Curves of generated from MiRXES and other vendors. MiRXES ID3EAL microRNA PCR system showed significantly earlier CT values emerging against other vendors across 5 different microRNA targets. Varying %AT was factored into each target to challenge the robustness of each system. Highly reproducible results between technical and biological replicates were generated with the use of ID3EAL microRNA qPCR mastermix

### High Reproducibility

MiRXES ID3EAL miRNA qPCR assays are optimally designed to generate high efficiency and reproducibility regardless of sample types. The technology was subjected to a concordance study between two independent laboratories over a year. 30 cancer sera were profiled for more than 200 microRNA targets over a year. The ease of use enables even first time users to generate consistent technical and biological replicates.

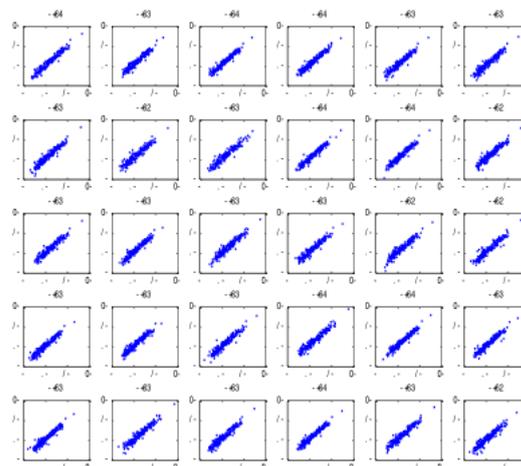


Figure 4. Comparison chart between two independent laboratories. 30 cancer sera were profiled for more than 200 microRNA. The data showed highly reproducible CT between the two laboratories ( $R^2 > 0.95$ )

## MiRXES ID3EAL Spike-In Kit

MiRXES ID3EAL Spike-Ins are synthetic miRNAs that have been designed with MiRXES' data driven proprietary thermodynamics model to minimize cross-over to endogenous miRNAs. MiRXES' Spike-Ins have been extensively tested and compatible with various isolation methods, including phenol/chloroform-based, phenol-free, membrane, bead and precipitation methods. It is critical that the method retains the small RNA fraction. MiRXES recommend users to use Spike-Ins with MiRXES' catalogued assays. MiRXES' Spike-In assays have been optimized together with MiRXES microRNA qPCR system.



Easy to incorporate solution to verify your microRNA workflow

Avoid time and reagent wastage situation due to poor sample quality

Highly flexible spike-ins to verify RNA extraction and reverse transcription processes

Varying spike-ins concentration allows confident assessment of extraction for a wide dynamic range of microRNA

Compatible with all most major microRNA extraction solution

## Performance of Spike-In

Extensive bioinformatics effort was invested to design MiRXES ID3EAL Spike-Ins synthetic microRNA. Coupled with MiRXES' proprietary ID3EAL primer design algorithm, it enables researchers to specifically detect spike-ins of samples with more than 600-plex. The samples also demonstrated extremely good linearity and high efficiency.

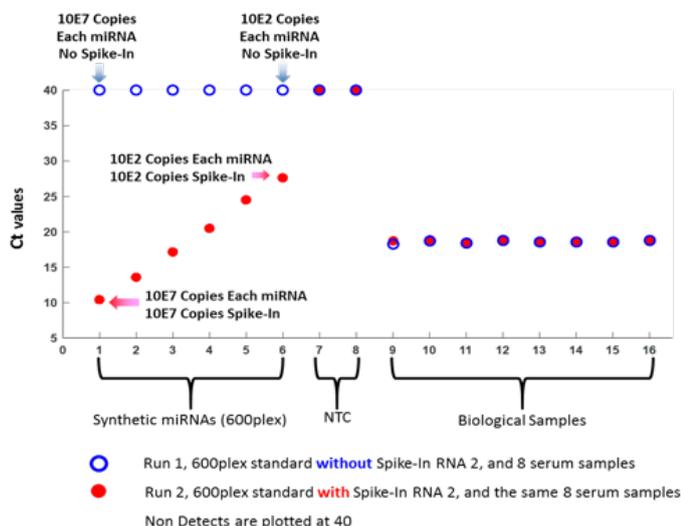


Figure 5. The graph shows Spike-In 2 tested on 16 samples for two times (Run 1: blue circle, Run 2: red dots). The first 6 samples are standard 10 fold dilutions comprised of 600 miRNA targets (600-plex) from 107 copies to 102 copies. Sample 7 and 8 are RT-NTC and qPCR NTC. In the first run, no Spike-In RNA was added; and in the second run, Spike-In RNA was added at 107 copies and diluted together with the 600-plex. In the first run, no signal was picked up by Spike-In primers while in the second run the Spike-In assay showed good linearity ( $x_2 > 0.99$ ) and efficiency of 1. The results proved Spike-In assay is specific and can be used to gauge workflow efficiency.

## Normalize your data with Spike-in Controls

It is critical to ensure that the quality and quantity of the RNA extracted from the biological source is optimal for downstream analysis such as qPCR. Spike-in controls may be incorporated in two part of the workflow - Isolation and Reverse Transcription. qPCR data points from these controls allow us to understand the efficiency of each part of the workflow. CT value higher than 30 indicates poor isolation or cDNA synthesis efficiency.

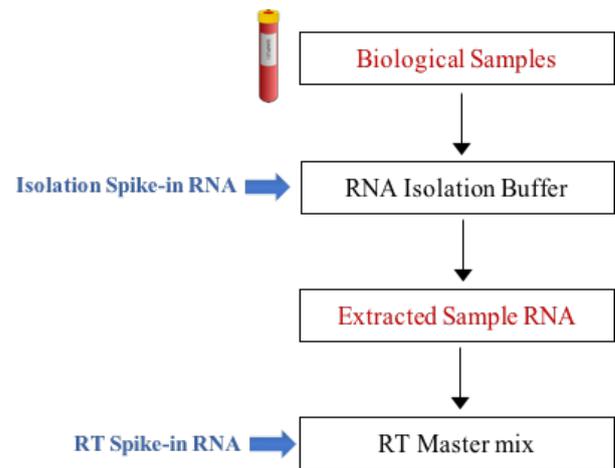


Table 1. Results interpretation for MiRXES ID3EAL Spike-In kit. The table below illustrates possible scenarios leading to the qPCR data generated from the kit. CT value lower than 30 indicates that the target can be detected and it is denoted as **x**. For value below 30, it indicates low expression and it is denoted as **✓**.

Controls	CT value higher than 30			
Spike-in controls for isolation	<b>x</b>	<b>✓</b>	<b>✓</b>	<b>x</b>
Spike-in controls for reverse transcription	<b>x</b>	<b>x</b>	<b>✓</b>	<b>x</b>
Normalizing strategy - reference genes	<b>✓</b>	<b>✓</b>	<b>✓</b>	<b>x</b>
	Low amount of microRNA in biological sample	Poor isolation efficiency	Isolation challenge and/or presence of inhibitors	ID3EAL for downstream analysis

## Application - Verification of Workflow

A test group of 74 Stem cell samples underwent microRNA isolation using the MiRXES ID3EAL Spike-In Kit. All the samples showed CT value below 30, with exception of Sample 48 that carries a higher CT values for both spike-in. This may suggest lower isolation and/or lower reverse transcription efficiency. MiRXES recommends normalizing with Spike-Ins first to eliminate process noises before proceed to other analysis.

Spike-In 1 and 2 added in Isolation, for 74 stem cell samples

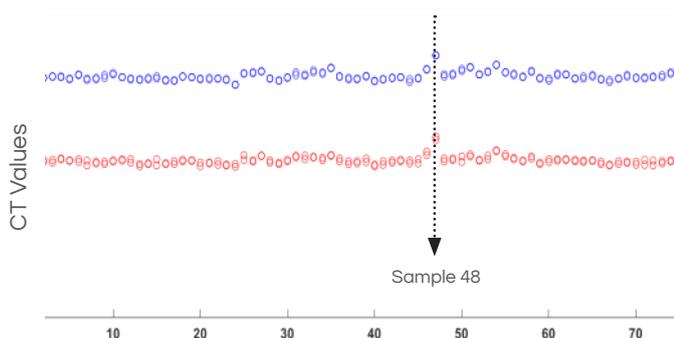


Figure 4. microRNA Isolation efficiency across 74 Stem cell samples. The graph shows the CT values generated from 74 stem cell samples isolated with spike-Ins. Red circles represent the CT values of Spike-In 1 of all 74 samples tested by 2 qPCR runs and blue circles represent the CT values of Spike-In 2 of all 74 samples tested by 2 qPCR runs. For each sample, the technical replicates of both Spike-In 1 and Spike-In 2 should be very tight (SD<0.5), or it may indicate experiment error. In the graph, Sample 48 showed a significantly lower Spike-In recovery manifested by its high CT values. MiRXES recommends normalizing with Spike-In first to eliminate process noise before proceed to other analysis.

Purchasing information

Product	Description	Cat. No.
ID3EAL miRNA qPCR Starter Kit, 2 Targets (20)	For 2 x targets, 20 cDNA and 100 qPCR reactions, 1 x 20 µl of ID3EAL Reverse Transcriptase (20x), 1 x 100 µl of ID3EAL miRNA RT Buffer (4x), 2 x 20 µl of ID3EAL miRNA RT Primers (20x), 1 ml of ID3EAL miRNA qPCR Master Mix (2x), 2 x 200 µl of ID3EAL miRNA qPCR Assay (10x), limited to human and mouse assays only	1104301
ID3EAL miRNA qPCR Starter Kit, 2 Targets -Hi-ROX (20)	For 2 x targets, 20 cDNA and 100 qPCR reactions, 1 x 20 µl of ID3EAL Reverse Transcriptase (20x), 1 x 100 µl of ID3EAL miRNA RT Buffer (4x), 2 x 20 µl of ID3EAL miRNA RT Primers (20x), 1 ml of ID3EAL miRNA qPCR Master Mix (2x), 2 x 200 µl of ID3EAL miRNA qPCR Assay (10x), limited to human and mouse assays only - For High-ROX qPCR instruments	1104371
ID3EAL Spike-in RNA Kit (50)	For 50 x 20 µl cDNA reactions and 200 qPCR reactions, 3 x 20 µl of ID3EAL Reverse Transcriptase (20x), 3 x 100 µl of ID3EAL miRNA RT Buffer (4x), 3 x 60 µl of ID3EAL miRNA RT Primers (20x), 1 x 250 µl of Pre-mixed Isolation Spike-in RNA, 2 ml of ID3EAL miRNA qPCR Master Mix (2x), 3 x 200 µl of ID3EAL miRNA qPCR Assay (10x)	1102103
ID3EAL Spike-in RNA Kit - Hi-ROX (50)	For 50 x 20 µl cDNA reactions and 200 qPCR reactions, 3 x 20 µl of ID3EAL Reverse Transcriptase (20x), 3 x 100 µl of ID3EAL miRNA RT Buffer (4x), 3 x 60 µl of ID3EAL miRNA RT Primers (20x), 1 x 250 µl of Pre-mixed Isolation Spike-in RNA, 2 ml of ID3EAL miRNA qPCR Master Mix (2x), 3 x 200 µl of ID3EAL miRNA qPCR Assay (10x) - For High-ROX qPCR instruments	1102173
ID3EAL Complete Spike-in RNA Kit (50)	For 50 x 20 µl cDNA reactions and 200 qPCR reactions, 3 x 20 µl of ID3EAL Reverse Transcriptase (20x), 3 x 100 µl of ID3EAL miRNA RT Buffer (4x), 3 x 60 µl of ID3EAL miRNA RT Primers (20x), 1 x 250 µl of Pre-mixed Isolation Spike-in RNA, 1 x 50 µl of Pre-mixed Reverse Transcription Spike-in RNA, 2 ml of ID3EAL miRNA qPCR Master Mix (2x), 3 x 200 µl of ID3EAL miRNA qPCR Assay (10x)	1102153
ID3EAL Complete Spike-in RNA Kit - Hi-ROX (50)	For 50 x 20 µl cDNA reactions and 200 qPCR reactions, 3 x 20 µl of ID3EAL Reverse Transcriptase (20x), 3 x 100 µl of ID3EAL miRNA RT Buffer (4x), 3 x 60 µl of ID3EAL miRNA RT Primers (20x), 1 x 250 µl of Pre-mixed Isolation Spike-in RNA, 1 x 50 µl of Pre-mixed Reverse Transcription Spike-in RNA, 2 ml of ID3EAL miRNA qPCR Master Mix (2x), 3 x 200 µl of ID3EAL miRNA qPCR Assay (10x) - For High-ROX qPCR instruments	1102173
ID3EAL Spike-in control for Reverse Transcription (50)	1 x 50 µl of Pre-mixed Reverse Transcription Spike-in RNA for 50 cDNA reactions	1102112
ID3EAL Spike-in control for Isolation (50)	1 x 250 µl of Pre-mixed Isolation Spike-in RNA for 50 cDNA reactions	1102122
ID3EAL cDNA Synthesis System (20)	For 20 x 20 µl cDNA reactions, 1 x 20 µl of ID3EAL Reverse Transcriptase (20x), 1 x 100 µl of ID3EAL miRNA RT Buffer (4x)	1103101
ID3EAL cDNA Synthesis System (60)	For 60 x 20 µl cDNA reactions, 3 x 20 µl of ID3EAL Reverse Transcriptase (20x), 3 x 100 µl of ID3EAL miRNA RT Buffer (4x)	1103103
ID3EAL Individual miRNA RT Primer 1-plex (20)	20 µl of ID3EAL miRNA RT Primer (20x)	1103111
ID3EAL Individual miRNA RT Primer 1-plex (60)	60 µl of ID3EAL miRNA RT Primer (20x)	1103113
ID3EAL Individual miRNA RT Primer 1-plex (100)	100 µl of ID3EAL miRNA RT Primer (20x)	1103114
ID3EAL Customized Individual miRNA RT Primer 1-plex - For non-human and mouse miRNA (500)	500 µl of ID3EAL miRNA RT Primer (20x)	1106104
ID3EAL Customized Individual miRNA RT Primer 1-plex - For non-miRBase miRNA (500)	500 µl of ID3EAL miRNA RT Primer (20x)	1106144
ID3EAL Multiplex miRNA RT Primers 96-plex (24)	2 x 24 µl of ID3EAL miRNA RT Primers 96-plex (10x)	1103151
ID3EAL Multiplex miRNA RT Primers 96-plex (72)	6 x 24 µl of ID3EAL miRNA RT Primers 96-plex (10x)	1103153
ID3EAL Individual miRNA qPCR Assay (100)	200 µl of ID3EAL miRNA qPCR Assay (10x)	1104101
ID3EAL Individual miRNA qPCR Assay (500)	1,000 µl of ID3EAL miRNA qPCR Assay (10x)	1104103
ID3EAL Customized Individual miRNA qPCR Assay - For non-human and mouse miRNA (500)	1,000 µl of ID3EAL miRNA qPCR Assay (10x)	1106173
ID3EAL Adv Customized Individual miRNA qPCR Assay - For non-miRBase miRNA (500)	1,000 µl of ID3EAL miRNA qPCR Assay (10x)	1106163
ID3EAL miRNA qPCR Master Mix (200)	2 x 1,000 µl of ID3EAL miRNA qPCR Master Mix (2x), 200 x 20 µl reactions	1104202
ID3EAL miRNA qPCR Master Mix (800)	8 x 1,000 µl of ID3EAL miRNA qPCR Master Mix (2x), 800 x 20 µl reactions	1104204
ID3EAL miRNA qPCR Master Mix (1200)	12 x 1,000 µl of ID3EAL miRNA qPCR Master Mix (2x), 1200 x 20 µl reactions	1104205
ID3EAL miRNA qPCR Master Mix (2400)	24 x 1,000 µl of ID3EAL miRNA qPCR Master Mix (2x), 2400 x 20 µl reactions	1104206
ID3EAL miRNA qPCR Master Mix - Hi-ROX (200)	2 x 1,000 µl of ID3EAL miRNA qPCR Master Mix (2x), 200 x 20 µl reactions, For High-Rox qPCR instruments	1104212
ID3EAL miRNA qPCR Master Mix - Hi-ROX (800)	8 x 1,000 µl of ID3EAL miRNA qPCR Master Mix (2x), 800 x 20 µl reactions, For High-Rox qPCR instruments	1104214
ID3EAL miRNA qPCR Master Mix - Hi-ROX (1200)	12 x 1,000 µl of ID3EAL miRNA qPCR Master Mix (2x), 1200 x 20 µl reactions, For High-Rox qPCR instruments	1104215
ID3EAL miRNA qPCR Master Mix - Hi-ROX (2400)	24 x 1,000 µl of ID3EAL miRNA qPCR Master Mix (2x), 2400 x 20 µl reactions, For High-Rox qPCR instruments	1104216

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# How to Choose a MiRNXES Product/Service

