

EPIK™ miRNA Panel and Select Assays

- **Increased sensitivity;** RT and qPCR steps optimized to drive highly efficient amplification from limiting amounts (≥ 1 pg) of input sample
- **Improved specificity;** pre-designed, novel, miRNA-specific primers for superior discrimination of even very closely related targets
- **Faster protocol;** RNA to Ct in just 2 hours for earlier results and increased throughput capacity
- **Reliable data;** 7-log linear dynamic range for accurate quantification of low- and high-expressed targets in even limited sample volumes
- **Convenience;** all necessary components included in the kit and formulated to minimize set-up time

EPIK™ miRNA Assays do not rely on sequence-dependent probes or chemically modified primers, instead they use modified stem-loop miRNA specific oligonucleotide mediated reverse transcription and hemi-nested real-time PCR, using SensiSMART™ and SYBR® Green to confer high sensitivity, specificity and speed, to the quantification of miRNAs.

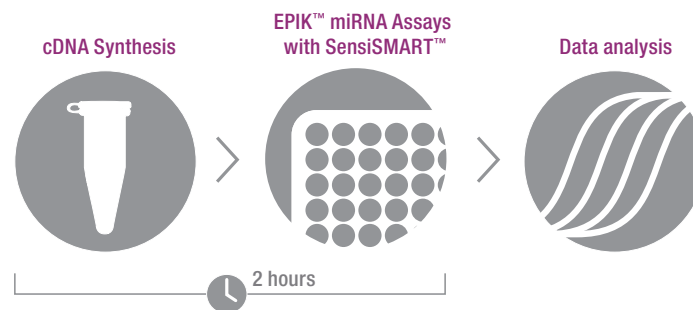


Fig. 1. Overview of workflow, from RNA to results in two hours

EPIK miRNA ASSAYS

Each assay comprises three miRNA specific primers designed using proprietary thermodynamic based algorithms from MiRXES

RT: Conformational restricted RT primer efficiently hybridizes to mature but not precursor form of the target miRNA

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

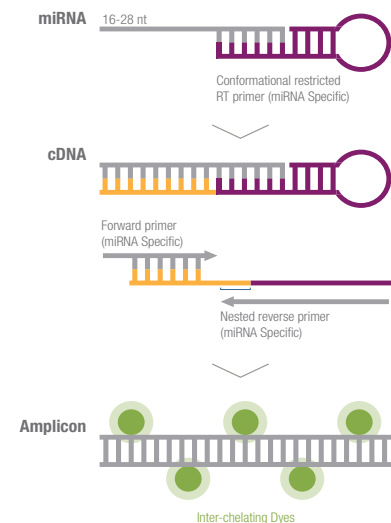


Fig. 2. EPIK™ miRNA assay workflow

SUPERIOR SENSITIVITY

EPIK miRNA RT-qPCR assays yield class-leading sensitivity when compared to other miRNA quantification systems (Fig. 3). Sensitive and robust detection of miRNAs with varying sequences gives confidence in profiling results.

miRNA	% AT	Ct value (10 ⁸ copies of templates)			
		EPIK miRNA	Supplier A	Supplier B	Supplier 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-232-3p	73%	13.0	21.1	21.2	N/A

Fig. 3A. Comparison of miRNA Assays

Comparison of five miRNAs with increasing AT concentrations between EPIK miRNA assay and three other suppliers.

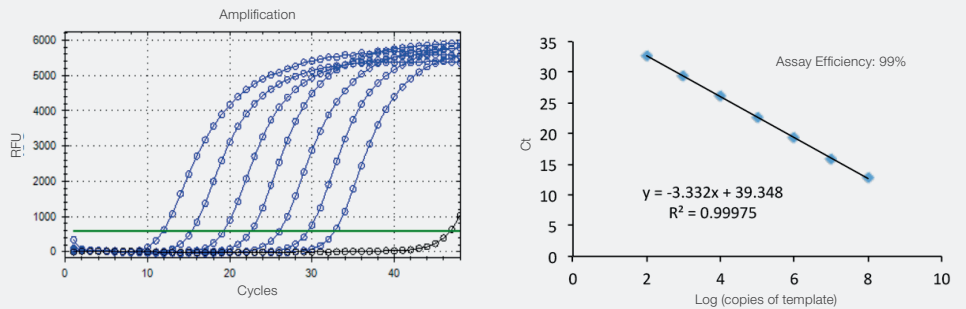


Fig. 3B. Performance of EPIK miRNA assay

Synthetic RNA of hsa-miR-99b-3p was reverse transcribed and amplified by EPIK miRNA assay. The results illustrate the sensitivity (10⁸ to 100 copies) and efficiency (99.97%) of the assay, allowing the detection of miRNAs at varying expression levels, including low expressers.

SPECIFICITY

Combination of miRNA specific RT primer and nested real-time PCR primer pairs enables EPIK miRNA assays to efficiently discriminate highly homologous miRNA family members with single nucleotide difference (Fig. 4.), giving virtually no cross-reactivity and so greater confidence in profiling results.

Relative Detection (%)		miRNA real-time RT-PCR Assay										
		let-7a	let-7b	let-7c	let-7d	let-7e	let-7f	let-7g	let-7i	miR-98		
U G A G G U A G	U A G G U U G	U A U A G U U	let-7a	100.0%	0.0%	0.1%	1.2%	0.0%	0.5%	0.0%	0.0%	
U G A G G U A G	U A G G U U G	U G U G G U U	let-7b	0.0%	100.0%	2.2%	0.0%	0.0%	0.0%	0.0%	0.0%	
U G A G G U A G	U A G G U U G	U A U G G U U	let-7c	0.8%	1.6%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
U G A G G U A G	U A G U U U G	U A U C G U U	let-7d	0.4%	0.0%	0.1%	100.0%	0.0%	0.0%	0.0%	0.0%	
U G A G G U A G	U A G A U U G	U A U A G U U	let-7e	0.1%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%	
U G A G G U A G	U A G U U U G	U A C A G U U	let-7f	0.1%	0.0%	0.0%	0.0%	0.1%	100.0%	0.0%	0.0%	
U G A G G U A G	U A G U U U G	U G C U G U U	let-7g	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	
A G A G G U A G	U A G G U U G	C A U A G U U	let-7i	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	
U G A G G U A G	G A G G U U G	U A U A G U U	miR-98	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%

Fig. 4. Relative detection across an miRNA family

Relative detection when assays were challenged with 10⁹ copies of mismatched targets from Let-7 family. The results show at least 50-fold specificity and virtually no cross-reaction.

REPRODUCIBILITY

EPIK miRNA RT-qPCR assays yielded highly reproducible results in profiling more than 200 miRNAs from 30 cancer sera over a year in two independent laboratories ($R^2 > 0.95$) (Fig. 5.). The ease of use enables even first time users to generate consistent technical and biological replicates.

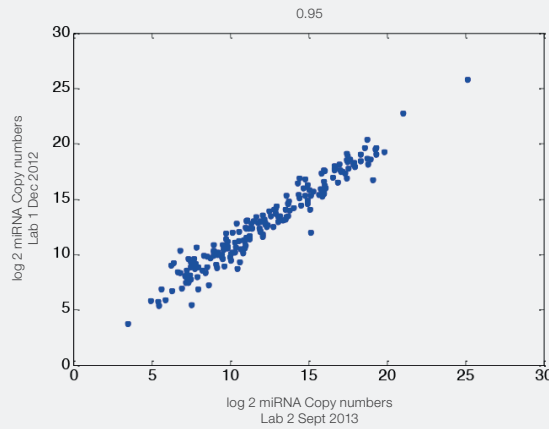
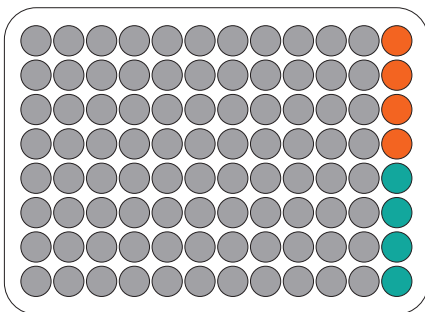


Fig. 5. 200 miRNAs from cancer sera
Comparison of 200 miRNA target amplifications in 30 cancer patient sera over a year in two independent laboratories. Measurement 1 (Y axis) was taken on Dec 2012 (the results of one is shown). Measurement 2 (X axis) was taken in Sep 2013. The results were in agreement and correlated with an R^2 value of 0.92-0.96 (depending on miRNA target).

EPIK miRNA SELECT ASSAYS

Each EPIK miRNA Select Assay Kit contains any four individual EPIK miRNA RT-qPCR assays (100 reactions per assay). Choose from between 1 and 4 individual EPIK Select Assays per assay kit (4 x 100 reactions) for the detection of any miRNAs in the miRBase.

EPIK miRNA Panel Assay



88 Panel miRNAs
2 spike-in miRNA
2 inter-plate calibrators

Panel	Panel miRNAs	96-well Plate per sample
Cancer	352	4
Biofluid	176	2
Stem Cell	176	2

KNOWLEDGE PANELS

miRNA knowledge panels are individual EPIK miRNA Select Assays assembled into biologically relevant panels based on state-of-the-art bioinformatics analysis of both in house data and published literatures.

- EPIK Cancer miRNA Panel:** research of the dysregulations and functions of miRNAs in various cancers account for close to half of all miRNA publications. Profiling of miRNA regulations could provide insights into cancer pathogenesis, drug response and recurrence.
- EPIK Stem Cell miRNA Panel:** stem cells are capable of both self-renewal and differentiation into multiple cell types. Understanding the critical roles of miRNAs in induction, maintenance, differentiation and reprogramming of stem cells helps to harness the great potential of stem cells as regenerative medicine.
- EPIK Biofluid miRNA Panel:** circulating miRNAs are now regarded as a promising new class of biomarkers and a novel means of extra-cellular communications.

Ordering Information

EPIK™ miRNA Select Assays	Size	Cat. #
EPIK miRNA Select Hi-ROX Kit	4 x 100 assays*	BIO-66046
EPIK miRNA Select Lo-ROX Kit	4 x 100 assays*	BIO-66045

EPIK™ miRNA Panel Assays	Size	Cat. #
EPIK Cancer miRNA Panel Lo-ROX Plate 0.1Y	8 Plates	BIO-66031
EPIK Cancer miRNA Panel Hi-ROX Plate 0.1Y	8 Plates	BIO-66032
EPIK Cancer miRNA Panel Lo-ROX Plate 0.2Y	8 Plates	BIO-66033
EPIK Cancer miRNA Panel Lo-ROX Plate 0.1X	8 Plates	BIO-66040
EPIK Stem Cell miRNA Panel Lo-ROX Plate 0.1Y	4 Plates	BIO-66034
EPIK Stem Cell miRNA Panel Hi-ROX Plate 0.1Y	4 Plates	BIO-66035
EPIK Stem Cell miRNA Panel Lo-ROX Plate 0.2Y	4 Plates	BIO-66036
EPIK Stem Cell miRNA Panel Lo-ROX Plate 0.1X	4 Plates	BIO-66041
EPIK Biofluid miRNA Panel Lo-ROX Plate 0.1Y	4 Plates	BIO-66037
EPIK Biofluid miRNA Panel Hi-ROX Plate 0.1Y	4 Plates	BIO-66038
EPIK Biofluid miRNA Panel Lo-ROX Plate 0.2Y	4 Plates	BIO-66039
EPIK Biofluid miRNA Panel Lo-ROX Plate 0.1X	4 Plates	BIO-66042

*Individual assay(s) specified during online order process

Please contact us for institutional pricing, special price quotations and availability of bulk pack sizes.



PSGBL1116V1.2

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