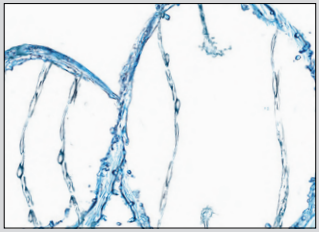

EPIK™ Stem Cell miRNA Panel Assay
EPIK™ Biofluid miRNA Panel Assay

Product Manual

BIOLINE

A Meridian Life Science® Company

EPIK™ miRNA Panel Assay

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GENERAL INFORMATION

1. KIT CONTENTS

EPIK™ Stem Cell/Biofluid miRNA ASSAY COMPONENTS	KIT	
Assay Plates*	4 x 96-well plates**	1 box
Stem Cell or Biofluid RT Primer Pool - lyophilized	2 tubes (A and B)	
RNA Spike - lyophilized	1 tube	
EPIK 5x RT Buffer	1 x 32 µl	1 box
EPIK RT Enzyme	1 x 8 µl	
2x SensiSMART™ SYBR Master Mix	4 x 1 ml	
DEPC Water	3 x 1.8 ml	

* For well definitions please see web site www.bioline.com/mirna
** Three 96-well configurations are available, depending on the real-time PCR machine used (see section 7)

2. DESCRIPTION

Mature microRNAs (miRNAs) are endogenously biosynthesized across many species of eukaryotes. These single-stranded RNAs (~ 22 nucleotides long) are known to play important regulatory roles in animals and plants by targeting mRNA transcripts for cleavage or translational repression. To date, thousands of unique, miRNAs have been identified (www.mirbase.org). Their expression levels vary greatly among species, tissues and in disorders.

Detecting miRNAs remains a significant challenge, mainly due to the short lengths of the nucleotide sequences. Various methods for miRNA measurement are currently available and quantitative real-time PCR remains the method of choice for both convenience and reliability. Stem-loop structure-based assays have been successfully used for the quantification of replicating viruses and mature miRNAs, however these methods rely on sequence-dependent probes or chemically modified primers for optimal specificity and are time-consuming, labour-intensive and suffer from sample-to-sample variability. There are many distinct advantages of EPIK™ miRNA Panel Assays over these miRNA-detection methods, including:

- Highly specific; targeting only mature miRNA and not precursors and can discriminate between highly similar miRNAs.
- Ultra-sensitive; can detect mature miRNA from as little as 10 pg of total RNA
- Wide dynamic range of quantitation; all assays can detect mature miRNAs with greater than six logs of dynamic range (1 million fold changes).
- Fast reaction time; the simple two-step protocol takes less than 2 hours from RNA to result.

The workflow of EPIK™ miRNA Panel Assays consists of 2 independent stages, where users have the choice of pausing between each stage (Fig 1).

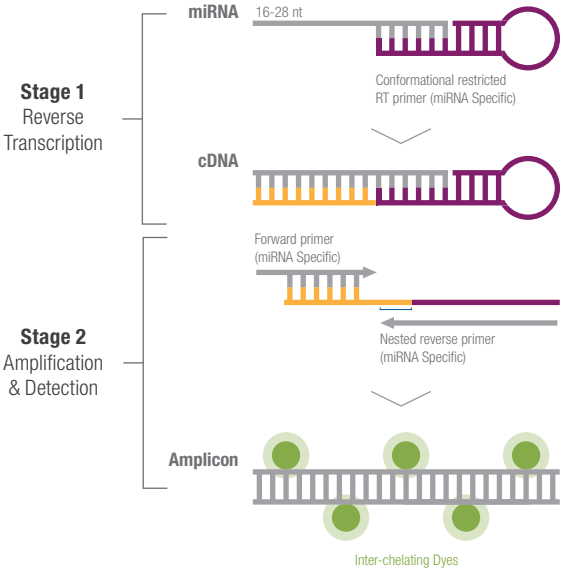


Fig. 1 EPIK™ miRNA Panel Assay powered by MiRXES™ technology
The EPIK™ miRNA Panel Assay comprises of a set of three specific primers. The conformationally-restricted RT primer allows efficient hybridization only to the mature form of the target miRNA (Stage 1). The miRNA-specific forward and nested reverse real-time PCR primers confer further specificity and enable robust amplification of the target cDNA (Stage 2).

All EPIK™ miRNA Panel Assays have been validated using both synthetic miRNA templates and total human RNA. Typically the assays detect as few as 100 copies of template per RT reaction with excellent assay efficiency and linearity (Fig. 2). These stem-loop structure-based assays are designed using MiRXES™ proprietary thermodynamics-based algorithms, enabling these assays to demonstrate superior sensitivities and specificities.

For the amplification and detection stage with SensiSMART™ qPCR mixes, a commonly available DNA-binding dye (SYBR Green) is used, rather than a probe-based system. This leads to remarkable sensitivities as well as extremely low background, enabling the accurate detection of very low miRNA levels. In addition, these assays, allow the clear discrimination between miRNA sequences with high similarity.

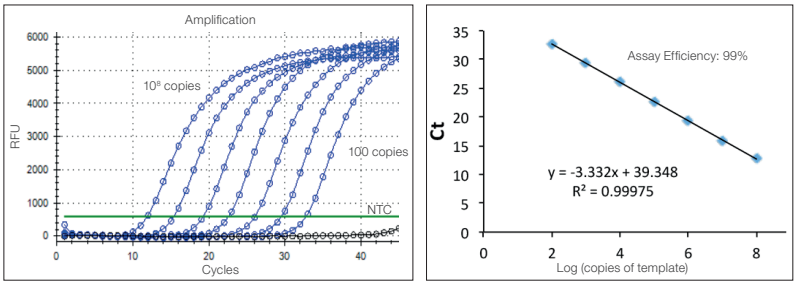


Fig. 2 Performance of EPIK™ miRNA Panel Assay for human miRNA.
A synthetic miRNA was reverse-transcribed and amplified by SensiSMART™. The results illustrate the sensitivity and efficiency (99.97%) of the assay, allowing the detection of miRNAs at varying expression levels (10^8 to 100 copies), including low expressers.

Please read this manual carefully to familiarize yourself with the EPIK™ miRNA Panel Assay protocol before starting (also available on www.bioline.com/mirna).

3. STORAGE

When stored under the recommended conditions and handled correctly, full activity of reagents is retained until the expiry date indicated on the outer box label.

Avoid subjecting any plate or reagent to repeated freezing and thawing. Reagents should be stored according to their label, with plates and the reagent box being stored at -20 °C and the RNA Spike stored at -80 °C after reconstitution.

4. SAFETY INFORMATION

When working with chemicals, always wear suitable personal protective equipment (PPE), including lab coat, gloves and safety glasses.

For detailed information, please consult the material data safety sheets (MSDSs) available on our website at www.bioline.com/mirna.

5. PRODUCT SPECIFICATIONS

EPIK™ miRNA Panel Assays are powered by MiRXES™ technology. The conformationally restricted RT primers are designed so that there is specific hybridization to the mature miRNA target. Following a reverse transcription stage, a robust amplification of the newly synthesized cDNA is accomplished using miRNA-specific forward and reverse real-time PCR primers to confer further specificity and sensitivity.

The EPIK™ miRNA Panel Assay protocol is optimized for use of up to 100 ng human total RNA per cDNA synthesis reaction (20 µl). The exact amount of human total RNA needed depends on the tissue state and can vary, depending on the type of cell, tissue or biofluid of interest and on the expression levels of the target miRNAs. As low as 10 pg of total RNA is sufficient for accurate quantification of highly expressed targets whereas up to 100 ng may be required for low expression miRNAs.

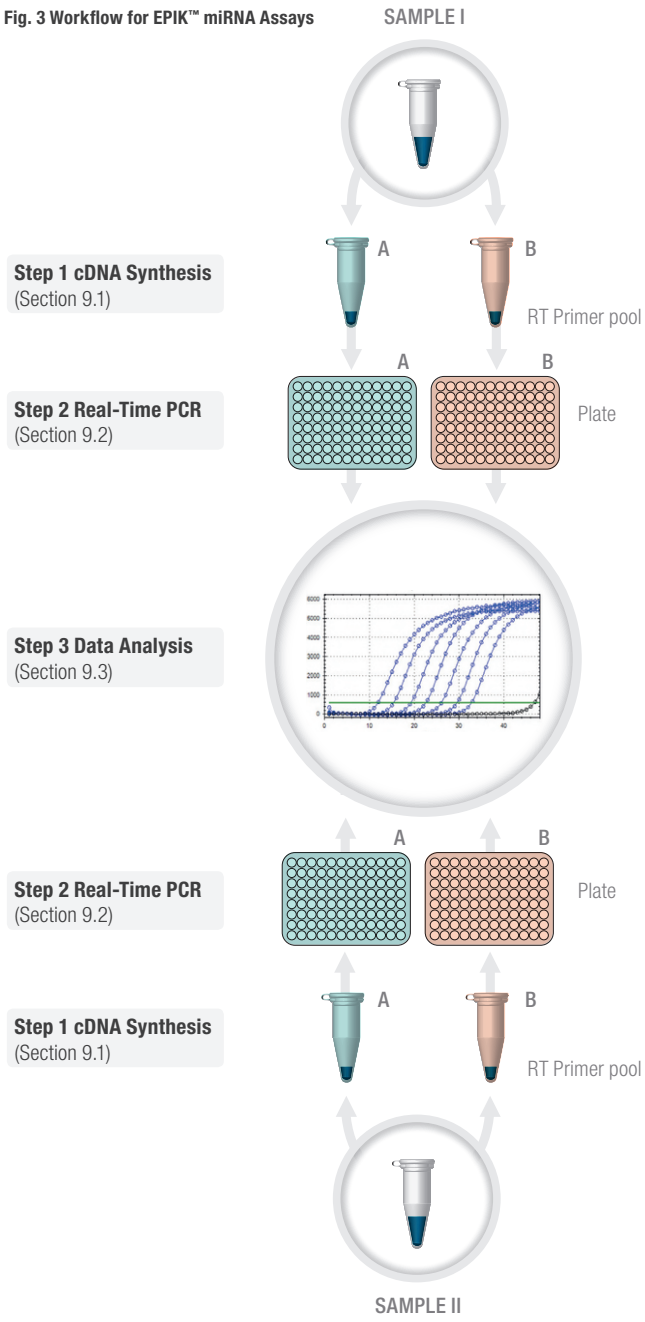
The qPCR primers for EPIK™ miRNA Panel Assays are supplied lyophilized in 96-well white plates, which are compatible with the machines listed in Section 7 (see www.bioline.com/mirna).

Plates with white wells provide improved signal-to-noise ratio in qPCR experiments and give the highest chance of detection of low-copy number[1] miRNA. The white plates supplied can be used in most machines without re-calibration and as a direct substitute for clear plates.

It is essential that the right plate type and the right reagent type are chosen for the machine you intend to use (see Section 7.2) (see www.bioline.com/mirna).

[1] See http://www.labnews.co.uk/features/never-break-the-chain/ and http://www.gene-quantification.de/qpcr2009/P025-qPCR-2009.pdf

Fig. 3 Workflow for EPIK™ miRNA Assays



6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY THE USER

The following additional items are required:

- Nuclease-free disposable plasticware
- Plate seals suitable for qPCR
- Microcentrifuge for 1.5 ml tubes
- Plate centrifuge suitable for 96-well plates
- Cooling block or ice bucket suitable for 96-well plates
- Heating block or thermocycler capable of isothermal heating at 42 °C and 70 °C
- Vortex

7. REAL-TIME PCR MACHINE AND ROX LEVEL

Please ensure that you have the correct plate type and SensiSMART™ ROX level for the machine you intend to run the assays on. Different qPCR machines from different manufacturers have specific requirements for the method of normalization employed, as well as for the plate size and shape. Using the wrong plate may damage your instrument (see www.bioline.com/mirna).

7.1. ROX

EPIK™ miRNA Panel Assays have been optimized for use in SYBR® Green-based real-time PCR on the real-time PCR instruments listed below, each of these instruments having the capacity to analyze the real-time PCR data with the passive reference signal either on or off.

- The EPIK™ Hi-ROX miRNA Panel Assays can be used on the ABI StepOne Plus.
- The EPIK™ Lo-ROX miRNA Panel Assays can be used on: ABI 7500, 7500 Fast QuantStudio® 3&5 Real-Time PCR System; QuantStudio™ 6 Real-Time PCR system; QuantStudio™ 7 Real-Time PCR system; QuantStudio™ 12K Flex Real-Time PCR system and ViiA7™. The EPIK™ Lo-ROX miRNA Panel Assays can also be used on the BioRad® CFX96 and Roche LightCycler® 480 that do not require the use of ROX.

7.2 96-well plates

The type of qPCR plate depends on which qPCR machine is to be used to run the experiment. The plates supplied will only fit machines fitted with a 96-well block. For advice on other plate types (384-well or 48-well) please contact Bioline Technical Support. We do not provide these assays for Rotor-Gene qPCR machines at the present time.

Due to the low volume of the qPCR reaction, we recommend the use of one of the following machines in combination with the following plate types:

Plate type 1 (0.1 ml, "low profile")†	ABI StepOne Plus; ABI ViiA7 FAST; ABI 7500 FAST; QuantStudio® 3 Real-Time PCR System FAST; QuantStudio® 5 Real-Time PCR System FAST; QuantStudio™ 6 Real-Time PCR system FAST; QuantStudio™ 7 Real-Time PCR system FAST; QuantStudio™ 12K Flex Real-Time PCR system FAST
Plate type 2 (0.2 ml)††	ABI ViiA7; ABI 7500; QuantStudio® 3 Real-Time PCR System; QuantStudio® 5 Real-Time PCR System; QuantStudio™ 6 Real-Time PCR system; QuantStudio™ 7 Real-Time PCR system; QuantStudio™ 12K Flex Real-Time PCR system
Plate type 3 (0.1 ml)	BioRad CFX96; Roche LC480 (96-well block only)

† This plate may also fit the following machines, though this operation is performed entirely at the users' own risk, and results may be variable. Bioline does not accept any responsibility for damage caused by using the incorrect plate type: ABI 7900HT FAST. Please contact Bioline Technical Support for more advice (see page 22).
†† This plate may also fit the following machines, though this operation is performed entirely at the users' own risk, and results may be variable. Bioline does not accept any responsibility for damage caused by using the incorrect plate type: ABI 7900HT; ABI 7300; ABI 7700; ABI 7000; BioRad iCycler IQ; BioRad IQ4; BioRad IQ5; BioRad Opticon; BioRad Chromo; Eppendorf Mastercycler ep realplex; Stratagene Mx3000; Stratagene MX3005; Stratagene MX4000; Techne Quantica. Please contact Bioline Technical Support for more advice (see page 22).

Each plate is supplied with dried down PCR primers specific for the following reactions:

- 88 miRNA-specific detection primer pairs (arrayed in columns 1 to 11, rows A to H)
- 2 RNA Spike control primers pairs (in duplicate) for the detection of the RNA Spike (column 12, rows A to D)
- 2 primer pairs (in duplicate) detecting artificial control DNA for plate-to-plate calibration (column 12, rows E to H)

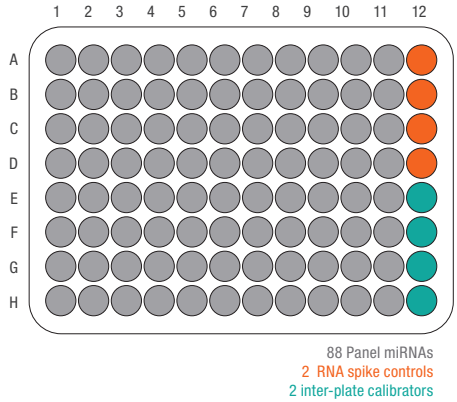


Fig. 4 EPIK™ miRNA Panel Plate layout
Wells A1 to H11 (red) each contain specific miRNA qPCR primers. Wells A12 and B12 contain replicate RNA Spike Control 1 (orange). Wells C12 and D12 contain replicate RNA Spike 2 (orange). Wells E12 and F12 contain replicate Inter-plate Calibrator 1 (green). Wells G12 and H12 contain replicate Inter-plate Calibrator 2 (green).

The EPIK™ Stem Cell miRNA Assay and EPIK™ Biofluid miRNA Assay both comprise of four plates. The miRNA most likely to be found in these types of samples (as shown in the published literature) are arrayed over plates A and B, to give a total of 176 separate miRNA-specific reactions. The 2 plates are supplied in duplicate so that the user can either:

- Perform a preliminary analysis of the experimental variation in one sample
- Or perform an initial screen against two conditions as a singlicate experiment (e.g. comparison of normal versus disease state)

8. IMPORTANT NOTES

Handle RNA carefully to avoid contamination by RNases, often found on labware, fingerprints and dust. For optimal RNA stability, keep RNA frozen at -20 °C for short-term or -80 °C for long-term storage.

It is important to work quickly when purifying RNA (see hints and tips on working with RNA at www.bioline.com/uk/rna-hints-and-tips).

8.1 Handling and storing starting material

We recommend using purified miRNA or total RNA with this panel rather than attempting direct detection of miRNA in partly purified sample types.

It is recommended that the ISOLATE II miRNA Kits are used for the preparation of the samples, as this allows rapid, unbiased, phenol-free isolation of miRNA.

The EPIK™ miRNA Panel Assay protocol is optimized for use of up to 100 ng total RNA per cDNA synthesis reaction (20 µl). Although the ratio between total RNA and specific miRNA is not fixed, measurement of total RNA provides a convenient way of estimating miRNA loading and an approximate methods for normalizing between experiments. If the ISOLATE II miRNA (BIO-52083) is used for example, the large RNA fraction concentration (as ng/µl), can be calculated from the final pure eluate and used as an estimate of total miRNA from the ISOLATE II miRNA column used to extract the miRNA fraction. This will only be correct if the elution volume of the large RNA fraction and miRNA fraction are identical.

8.2 Controls and calibrators

The RT-qPCR miRNA detection procedure outlined in this manual includes a synthetic RNA control (RNA Spike) which provides an accurate and convenient internal control for the experiment (for the suitability of such controls, see Redshaw *et al* (2013) Biotechniques **54**: 155-164 and Ho *et al* (2013) PLoS One **8**: e72463.

The RNA Spike once reconstituted from its lyophilized state can be used in two modes:

- as an RNA spike to be used during the sample isolation, i.e. added to the lysis buffer of the RNA isolation kit: after reconstitution in 30 µl of nuclease-free water, 5 µl should be used per sample isolation and this can be used to detect differences in RNA purification.
- an RT control for pre-purified miRNA, i.e. added during the reverse transcription stage: once reconstituted in 30 µl of nuclease-free water, addition of the RNA Spike in the reverse transcription stage (1 µl per RT reaction) will allow normalization between RT reactions, in the same way as reference genes are used in qPCR measurements of mRNA.

Users should not attempt to use RNA Spike in both modes in the same experiment.

If the user wishes to use their own calibration or plate-to-plate calibrator, then column 12 can be used to aliquot user-supplied primers. Control primers sets supplied in the 96-well plate target artificial sequences with no known homology to natural or artificial sequences deposited in miRBase (release 21, June 2014). As optimisation is required to ensure that user controls function properly, we recommend using the controls supplied.

8.3 Analysis

For convenience an Excel spreadsheet can be downloaded from the product page at www.bioline.com/mirna, which can be used to analyse plate to plate variation as well as for analysis and presentation of the data.

9. PROTOCOL

The EPIK™ miRNA Panel Assays protocol is a two-step protocol consisting of:

Step 1.	Reverse transcription with miRNA-specific RT-oligonucleotides and EPIK™ cDNA synthesis kit - See section 9.1
Step 2.	Real-Time PCR using SensiSMART™ Master Mix and amplification primers - See section 9.2

It is critical for the success of the experiment to follow the protocol carefully, from first-strand cDNA synthesis to real-time PCR amplification (approximately 2 hours). However, the procedure can be paused after the first-strand cDNA synthesis and the undiluted cDNA may be stored at -20 °C for up to three days.

Workflow

When working with the EPIK™ Stem Cell or Biofluid miRNA Panel Assay, it can be difficult for a single user with a single qPCR machine to run all the plates in one day. We suggest that in order for all the panels to be treated the same, storage should occur just after cDNA synthesis (see step 9.1.6). All the cDNA reactions must be treated identically, so if it is not possible to run all the plates within one day, all the cDNA reactions must be frozen at -20 °C once completed. This will ensure that all cDNA reactions are subjected to the same number of freeze-thaw cycles.

When a qPCR machine is available, remove a cDNA reaction from storage and prepare the qPCR as detailed in section 9.2. Thaw the qPCR master mix and cDNA as late as possible before running the qPCR. Once mixed (step 9.2.2), the cDNA-qPCR master mix should be loaded into the PCR plate and run on the instrument immediately. Storage of the ready to run plate at this point, even on ice, may result in non-specific primer-dimer formation.

When the first run is nearly finished, remove the next cDNA reaction tube and repeat the process with the next plate.

The remaining tubes and plates can be deployed in a similar way. The user should allow sufficient time so that all the real-time data can be collected in as short a time as possible, as we recommend that the cDNA from step 9.1.6 is stored at -20 °C for no more than three days. It should be possible for a single user with a single qPCR machine to run all the samples within 72 hours.

In order to minimize the effect of -20 °C storage, we recommend processing the plates of the same panel as close as possible in time, running successively panels A and B.

For more details and information on workflows please refer to section 9.4.

9.1 First-strand cDNA synthesis (step 1)

It is important to keep the components and the reactions on ice during the procedure.

9.1.1 Prepare template RNA

Gently thaw template RNA on ice. We recommend the use of 100 ng or less of total RNA per 20 µl RT reaction (see section 8.1). It is important to ensure that the same amount of RNA is used across the different panels. Adjust each of the template RNA samples to similar concentration using nuclease free water.

9.1.2 Prepare reagents

1. Reconstitute the RNA spike (the synthetic miRNA control) by adding 30 µl of nuclease-free water to the tube and vortexing. Incubate at room temperature for five minutes. Spin down in a microcentrifuge, then re-vortex for at least thirty seconds.
2. Reconstitute the Stem Cell or Biofluid RT Primer Pools (A and B) by adding 20 µl of nuclease-free water to the tube and vortexing. Incubate at room temperature for five minutes. Spin down in a microcentrifuge, then re-vortex for at least thirty seconds. Store at -20 °C.
3. Gently thaw the EPIK 5x RT Buffer and Stem Cell or Biofluid RT Primer Pool tubes on ice. Mix by vortexing (1 second) and spin down.
   Note: In case of precipitate in the EPIK 5x RT Buffer, incubate at 37 °C and vortex.

9.1.3 Assemble reagents

For analysis of 176 miRNAs in both the Stem Cell and Biofluid Panels, 2 RT reactions are required per sample, using RT primer A and B.

Assemble the reaction as indicated in Table 1: The most consistent results can be obtained by preparing a mastermix with template RNA, EPIK 5x RT buffer, water and EPIK RT enzyme in the proportions shown. The EPIK RT Enzyme should be added to the master mix last, right before dispensing of the master mix into sample tubes.

Dispense 2 µl of each primer pool into 2 separate tubes, then add 18 µl of the master mix.
Prepare 2 reverse transcription reactions as follows:

Table 1.

Reagent	Volume (Tube A)	Volume (Tube B)
Template RNA	X µl (up to 6 µl)	X µl (up to 6 µl)
EPIK 5x RT Buffer	4 µl	4 µl
DEPC water	6 µl - X µl	6 µl - X µl
EPIK RT Enzyme	1 µl	1 µl
Stem Cell or Biofluid RT Primer Pool A	2 µl	-
Stem Cell or Biofluid RT Primer Pool B	-	2 µl
Total volume	**20 µl**	**20 µl**

9.1.4 Mix and spin

Thoroughly mix the reagents by gently pipetting up and down. Spin down after mixing.

9.1.5 Incubate and heat inactivate

Incubate reaction at 42 °C for 30 min, followed by heat-inactivation of the reverse transcriptase at 90 °C for 5 min. Keep the undiluted cDNA reactions on ice until the assembly of real-time PCR reaction, or go to step 9.1.6 for storage.

9.1.6 Store cDNA

If desired, undiluted cDNA reactions can be stored at -20 °C for up to three days. It is recommended to store cDNA in “low-nucleic acid binding” (pre-siliconized) tubes.

9.2 REAL-TIME PCR AMPLIFICATION AND DETECTION (Step 2)

In this step, the cDNA is amplified by real-time PCR in the EPIK™ Stem Cell or Biofluid miRNA Panel plate.

Do not remove the plate from the sealed bag provided until you are ready to perform the qPCR step for that plate. If multiple qPCR machines are not available, see section 9.4.1 for suggestions for workflow.

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

9.2.1 Prepare reagents

1. Thaw 2x SensiSMART™ PCR Master Mix and cDNA reactions (A or B) as required on ice (see section 9.4).
2. Mix by quickly vortexing and spin down. cDNA reactions A and B are to be used with PCR plate A and B respectively (see Fig. 3).

9.2.2 Assemble the real-time PCR reagents

1. Mix each cDNA library with 2x SensiSMART™ PCR Master Mix and nuclease-free water in the proportion indicated in Table 2. Mix by vortexing and spin down.

Table 2.

Reagent	Volume (Plate A)	Volume (Plate B)
2x SensiSMART™ PCR Master Mix*	1000 µl	1000 µl
DEPC water	980 µl	980 µl
cDNA reaction A	20 µl	-
cDNA reaction B	-	20 µl
Total volume	**2000 µl**	**2000 µl**

* It is critical to place the PCR plate on a cooling block or on ice throughout the procedure.

2. Remove the plate from the blue foil bag and place on a cold block or in an ice bucket.
3. Carefully peel back the carrier seal from the top of the plate and discard.
4. Dispense 20 µl cDNA:PCR master mix per well (see Table 2) into the corresponding PCR plate.
5. Seal the plate using a qPCR-compatible seal.

9.2.3 Mix and spin

Centrifuge the plate briefly (30 s at 200 x g in a suitable plate centrifuge).

9.2.4 Real-Time PCR amplification

Perform real-time PCR amplification according to the following cycling parameters.

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

We recommend adding a melt-curve analysis step to your reaction conditions. This is normally added as a set module during qPCR machine programming and recommendations vary between manufacturers. Please refer to the manufacturer's machine-specific manual for more advice.

To obtain accurate, specific results for the miRNA and control assays, you must ensure that the real-time PCR amplification is performed exactly as set out above. Deviation from the protocol will yield poor results.

9.2.5 Data collection

Collect raw Ct values (also known as Cp or Cq, depending on the PCR instrument) using the software supplied with the real-time PCR instrument. Please note that it is not recommended to use auto Ct settings, but set the threshold manually to one tenth of the average maximal fluorescence value. We recommend that you export the data as an Excel file for further analysis.

9.3 ANALYSIS OF RESULTS

9.3.1 Verifying the controls

The Ct values in wells E12 and F12 and wells G12 and H12 of each plate (see figure 4 or plate layout) should be approximately the same. Variation between wells replicates of the same sample on the same plate can also give an estimation of experimental error.

It is possible to use the panels as a “plus-minus” screen in order to determine which miRNAs are present or absent from the sample.

The RNA Spike is detected by two distinct assays. When the RNA Spike is used as a control, please ensure that the RNA Spike Ct values from the following wells are similar:

- Wells A12 and B12
- Wells C12 and D12

Variation between these duplicates gives a measure of the experimental error due to pipetting.

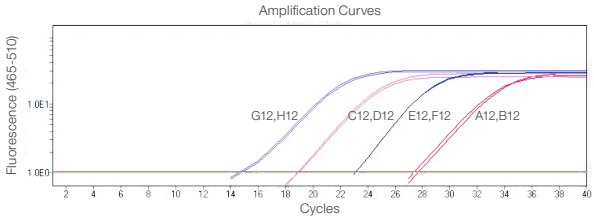


Fig. 5 EPIK™ miRNA Panel Plate calibrators
Amplification plot showing the inter-plate calibrators (Calibrator 1 - wells E12 and F12 and Calibrator 2 - wells G12 and H12) and spike controls (Spike Control 1 - wells A12 and B12 and Spike Control 2 - wells C12 and D12) from the EPIK™ Panel Assay. The results show very little variation between the duplicates, indicating low pipetting errors.

9.3.2 miRNA profile analysis (relative presence or absence)

The most powerful application of the miRNA panels is when the results are used to make an estimate of abundance of each miRNA relative to a control. The panels supplied here allow the simultaneous relative determination of hundreds of miRNA species, either in duplicate with a single sample, or as single-panel assays of two samples. Raw data can then be exported into the Excel spreadsheet provided on our EPIK™ miRNA web page for further analysis (see www.bioline.com/mirna).

The user can deploy the software supplied with their qPCR machine. Some manufacturers supply software which is powerful enough to determine well-to-well, plate-to-plate and sample-to-sample variation.

During the analysis, we recommend that at Ct of less than 36 is taken to be a positive reaction and Ct of more than 36 is taken to be negative. Some caution should be applied when examining miRNA with a Ct of 34 and above.

9.3.3 Absolute quantification of miRNA

These miRNA assays are only suitable for determining relative abundance. The format of the panels does not allow an analysis of the absolute number of miRNA molecules in the sample. These measurements are complex to perform and to establish the limitations of the technology, users should consult the literature on the subject. In order to determine efficiency and other essential parameters critical for absolute determination, users should request individual miRNA assays in larger quantities. Please contact Bioline for more details (see page 22).

9.4 RECOMMENDED WORKFLOW

Please see the comments made on workflow at the beginning of this section (9).

9.4.1 Using a single qPCR machine

1. The same qPCR machine type should be used to perform all of the qPCR cycling and analysis. While some plate types will fit in qPCR machines from different manufacturers, you are at risk of damaging your qPCR machine by using the wrong plate type. In addition, data from different makes of qPCR machines are processed using different methods. Using multiple manufacturers' qPCR machines will make your analysis more complicated.

2. Perform the cDNA synthesis (see step 9.1.6).

3. Store all the cDNA reactions at -20 °C once complete.

4. When the qPCR machine is available, remove the first cDNA reaction tube and thaw gently on ice. This storage step ensures that all the samples have been treated in a similar way, in that all the samples are subjected to one cycle of freeze/thaw.

5. Remove the first cDNA reaction tube from ice and perform steps 9.2.1 and 9.2.2. Make sure that the plate is sealed with a qPCR-compatible plate seal.

6. Place the plate in the qPCR machine and run the qPCR program.

7. Once prepared, plates should NOT be frozen at -20 °C. Plates should be processed immediately after preparation.

8. Start preparation of the next plate so that it is ready to be placed in the qPCR machine immediately after all the data from the first plate has been collected. We recommend starting 15-20 min before the end of the run.

9. Repeat steps 1 to 8 with the remaining plates. There are 4 plates for both the Biofluid and Stem Cell panels and 8 plates in total for the Cancer panel.

   The user should allow sufficient time so that all the real-time data can be collected in as short a time as possible. We recommend that the cDNA from step 9.1.6 is stored at -20 °C for no more than three days. It should be possible for a single user with a single qPCR machine to run all the samples within 72 hours.

9.4.2 Using multiple qPCR machines

If the user has multiple identical qPCR machines, all the reactions (RT and qPCR) should be performed in parallel. Take steps to ensure that all the RT reactions and qPCR reactions are treated identically. You should make sure that the same number of freeze/thaw steps is applied across replicates.

A TECHNICAL SUPPORT AND TROUBLESHOOTING

For technical assistance or more information on these products, please email us at tech@bioline.com

B ASSOCIATED PRODUCTS

Product	Size	Cat. #
ISOLATE II miRNA Kit	25 prep	BIO-52083
ISOLATE II RNA/DNA/Protein Kit	50 prep	BIO-52085
ISOLATE II Biofluid Kit	50 prep	BIO-52086
ISOLATE II FFPE RNA/DNA Kit	50 prep	BIO-52087

C PRODUCT WARRANTY AND DISCLAIMER

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

D TRADEMARK AND LICENSING INFORMATION

1. Trademarks: SensiSMART™, EPIK™ (Bioline Reagents Ltd), SYBR® (Molecular Probes), StepOne™, QuantStudio®, ViiA7™ (ABI), Mx4000, Mx3000P and Mx3005P (Stratagene), iCycler™, MyiQ5™, Opticon™, Chromo4™, Miniopticon™, (Bio-Rad), LightCycler®, ROX™ (Roche), RealPlex™ (Eppendorf), MX4000 (Stratagene).

2. Purchase of this product includes limited right to use the supplied amount of SYBR® Green I stain patented by Molecular Probes, Inc.

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6. The technology employed in this product is covered by Patent No: 185776, SG. Patents pending in other nations.

Ordering Information

Product	Size	Cat. #
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

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