miRStar™ Human Cancer Focus miRNA PCR Array

Cat#: AS-MR-001

Instruction Manual version 1.0

Product Summary

Kit components

Catalog Number	Contents	Storage
AS-MR-001	miRStar™ Human Cancer Focus miRNA PCR Array, dried down assays in 384-well plate	-20°C

Description

Arraystar's miRStar™ Human Cancer Focus microRNA PCR Array contains two identical sets of 184 miRNAs most relevant to cancer. Two individual samples, for example tumor vs. peritumoral or biological replicates, can be profiled in parallel. To ensure high data quality, the panel includes 8 miRNA reference sets to better quantify and normalize the qPCR data. cDNA synthesis and PCR efficiency are evaluated by using the synthetic cel-miR-39-3p as the Spike-in RNA control. The array is a powerful tool to conveniently and quickly analyze the expression levels of miRNAs most relevant to cancers, which is valuable for cancer biology research and cancer biomarker discovery.

Array Layout

The cancer-associated miRNAs for each sample are in the alternate rows (shaded in light or darker colors for Sample 1 and Sample 2). The control assays are circled in red.

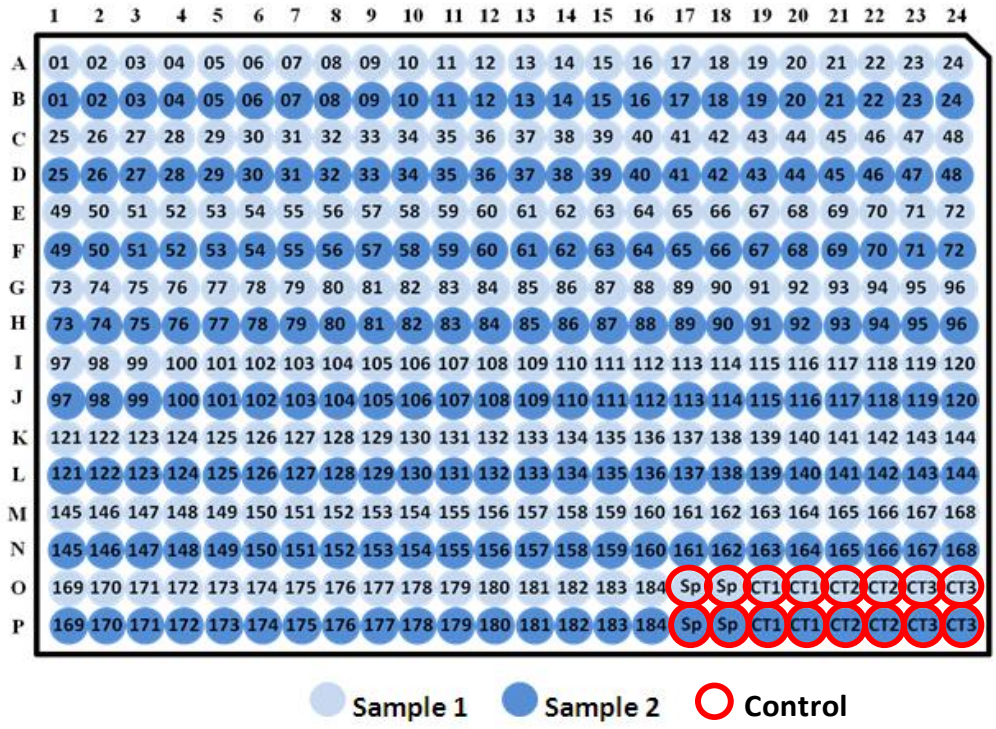

Figure 1. The array layout for miRStar Human Cancer Focus miRNA PCR Array.

#01 through #184	184 cancer-associated miRNAs.
O17 and O18 P17 and P18	Spike-in Control (**SP**) in duplicate, to evaluate cDNA synthesis and PCR efficiency.
O19 through O24 P19 through P24	Three small nuclear or small nucleolar RNAs in duplicates, RNU6-2-F (**CT1**), SNORD43-F (**CT2**), and SNORD95-F (**CT3**), to normalize qPCR data for the miRNAs.
#51, #66, #144, #146, #177	Five housekeeping miRNAs as the internal quantification controls or reference genes: hsa-miR-16-5p(#51, Well E03 and F03), hsa-miR-191-5p(#66, Well E18 and F18), has-miR-423-3p(#144, Well K24 and L24),

hsa-miR-425-5p(#146, Well M02 and N02), and hsa-miR-93-5p(#177, Well O09 and P09).

Description of Control Assays

There are three types of control assays built in the miRStar Human Cancer Focus miRNA PCR Array 384HC. Each control assay is in duplicate. Their uses and meanings are explained below.

- **SP** (Spike-in control): An RNA spike-in control is added in the RNA sample during the first-strand cDNA synthesis (Protocol Step A2). The SP control assay indicates the overall success and the efficiency of the reactions beginning from the adaptor ligation, cDNA synthesis to the final qPCR. Any problem(s) in these steps will result in a failed or compromised SP outcome.
- **CT** (miRNA Control Reference): Three stably expressed small nuclear or small nucleolar RNA genes RNU6-2-F (**CT1**), SNORD43-F (**CT2**), and SNORD95-F (**CT3**) are included in the array as the quantification references for miRNA. Additionally,
- **Housekeeping miRNA genes**: Five housekeeping miRNAs, namely, hsa-miR-16-5p(#51, Well E03 and F03), hsa-miR-191-5p(#66, Well E18 and F18), has-miR-423-3p(#144, Well K24 and L24), hsa-miR-425-5p(#146, Well M02 and N02), and hsa-miR-93-5p(#177, Well O09 and P09), can also serve as the internal quantification or reference controls.

List of miRNAs and controls

Control RNAs are outlined in red.

A01	let-7a-5p	B01	let-7a-5p	E01	miR-15a-5p	F01	miR-15a-5p	I01	miR-25-3p	J01	miR-25-3p	M01	miR-424-5p	N01	miR-424-5p
A02	let-7b-5p	B02	let-7b-5p	E02	miR-15b-5p	F02	miR-15b-5p	I02	miR-26a-5p	J02	miR-26a-5p	M02	miR-425-5p	N02	miR-425-5p
A03	let-7b-3p	B03	let-7b-3p	E03	miR-16-5p	F03	miR-16-5p	I03	miR-26b-5p	J03	miR-26b-5p	M03	miR-429	N03	miR-429
A04	let-7c	B04	let-7c	E04	miR-17-5p	F04	miR-17-5p	I04	miR-27a-3p	J04	miR-27a-3p	M04	miR-451a	N04	miR-451a
A05	let-7d-5p	B05	let-7d-5p	E05	miR-181a-5p	F05	miR-181a-5p	I05	miR-27b-3p	J05	miR-27b-3p	M05	miR-486-5p	N05	miR-486-5p
A06	let-7d-3p	B06	let-7d-3p	E06	miR-181b-5p	F06	miR-181b-5p	I06	miR-28-5p	J06	miR-28-5p	M06	miR-495-3p	N06	miR-495-3p
A07	let-7e-5p	B07	let-7e-5p	E07	miR-181c-5p	F07	miR-181c-5p	I07	miR-299-3p	J07	miR-299-3p	M07	miR-497-5p	N07	miR-497-5p
A08	let-7f-5p	B08	let-7f-5p	E08	miR-182-5p	F08	miR-182-5p	I08	miR-29a-3p	J08	miR-29a-3p	M08	miR-501-5p	N08	miR-501-5p
A09	let-7g-5p	B09	let-7g-5p	E09	miR-183-5p	F09	miR-183-5p	I09	miR-29a-5p	J09	miR-29a-5p	M09	miR-502-3p	N09	miR-502-3p
A10	let-7i-5p	B10	let-7i-5p	E10	miR-184	F10	miR-184	I10	miR-29b-3p	J10	miR-29b-3p	M10	miR-505-3p	N10	miR-505-3p
A11	miR-1	B11	miR-1	E11	miR-185-5p	F11	miR-185-5p	I11	miR-29b-2-5p	J11	miR-29b-2-5p	M11	miR-517a-3p	N11	miR-517a-3p
A12	miR-100-5p	B12	miR-100-5p	E12	miR-186-5p	F12	miR-186-5p	I12	miR-29c-3p	J12	miR-29c-3p	M12	miR-518a-3p	N12	miR-518a-3p
A13	miR-101-3p	B13	miR-101-3p	E13	miR-187-5p	F13	miR-187-5p	I13	miR-30a-5p	J13	miR-30a-5p	M13	miR-518b	N13	miR-518b
A14	miR-106a-5p	B14	miR-106a-5p	E14	miR-188-5p	F14	miR-188-5p	I14	miR-30b-5p	J14	miR-30b-5p	M14	miR-518c-3p	N14	miR-518c-3p
A15	miR-106b-5p	B15	miR-106b-5p	E15	miR-18a-5p	F15	miR-18a-5p	I15	miR-30c-5p	J15	miR-30c-5p	M15	miR-518e-3p	N15	miR-518e-3p
A16	miR-107	B16	miR-107	E16	miR-18b-5p	F16	miR-18b-5p	I16	miR-30d-5p	J16	miR-30d-5p	M16	miR-518f-3p	N16	miR-518f-3p
A17	miR-10a-5p	B17	miR-10a-5p	E17	miR-190a	F17	miR-190a	I17	miR-30e-5p	J17	miR-30e-5p	M17	miR-519d	N17	miR-519d
A18	miR-10b-5p	B18	miR-10b-5p	E18	miR-191-5p	F18	miR-191-5p	I18	miR-30e-3p	J18	miR-30e-3p	M18	miR-524-5p	N18	miR-524-5p
A19	miR-122-5p	B19	miR-122-5p	E19	miR-192-5p	F19	miR-192-5p	I19	miR-31-5p	J19	miR-31-5p	M19	miR-532-5p	N19	miR-532-5p
A20	miR-125a-5p	B20	miR-125a-5p	E20	miR-193b-3p	F20	miR-193b-3p	I20	miR-32-5p	J20	miR-32-5p	M20	miR-539-5p	N20	miR-539-5p
A21	miR-125b-5p	B21	miR-125b-5p	E21	miR-195-5p	F21	miR-195-5p	I21	miR-320a	J21	miR-320a	M21	miR-584-5p	N21	miR-584-5p
A22	miR-126-3p	B22	miR-126-3p	E22	miR-196a-5p	F22	miR-196a-5p	I22	miR-323a-3p	J22	miR-323a-3p	M22	miR-617	N22	miR-617
A23	miR-127-3p	B23	miR-127-3p	E23	miR-196b-5p	F23	miR-196b-5p	I23	miR-324-3p	J23	miR-324-3p	M23	miR-629-5p	N23	miR-629-5p
A24	miR-130a-3p	B24	miR-130a-3p	E24	miR-197-3p	F24	miR-197-3p	I24	miR-326	J24	miR-326	M24	miR-652-3p	N24	miR-652-3p
C01	miR-130b-3p	D01	miR-130b-3p	G01	miR-199a-5p	H01	miR-199a-5p	K01	miR-328	L01	miR-328	O01	miR-7-5p	P01	miR-7-5p
C02	miR-132-3p	D02	miR-132-3p	G02	miR-19a-3p	H02	miR-19a-3p	K02	miR-331-3p	L02	miR-331-3p	O02	miR-744-5p	P02	miR-744-5p
C03	miR-133a	D03	miR-133a	G03	miR-19b-3p	H03	miR-19b-3p	K03	miR-335-5p	L03	miR-335-5p	O03	miR-877-5p	P03	miR-877-5p
C04	miR-133b	D04	miR-133b	G04	miR-200a-3p	H04	miR-200a-3p	K04	miR-339-5p	L04	miR-339-5p	O04	miR-885-5p	P04	miR-885-5p
C05	miR-134	D05	miR-134	G05	miR-200b-3p	H05	miR-200b-3p	K05	miR-33a-5p	L05	miR-33a-5p	O05	miR-886-3p	P05	miR-886-3p
C06	miR-136-5p	D06	miR-136-5p	G06	miR-202-3p	H06	miR-202-3p	K06	miR-33b-5p	L06	miR-33b-5p	O06	miR-9-5p	P06	miR-9-5p
C07	miR-137	D07	miR-137	G07	miR-203a	H07	miR-203a	K07	miR-340-5p	L07	miR-340-5p	O07	miR-92a-3p	P07	miR-92a-3p
C08	miR-139-5p	D08	miR-139-5p	G08	miR-204-5p	H08	miR-204-5p	K08	miR-342-3p	L08	miR-342-3p	O08	miR-92b-3p	P08	miR-92b-3p
C09	miR-140-5p	D09	miR-140-5p	G09	miR-205-5p	H09	miR-205-5p	K09	miR-345-5p	L09	miR-345-5p	O09	miR-93-5p	P09	miR-93-5p
C10	miR-141-3p	D10	miR-141-3p	G10	miR-208a	H10	miR-208a	K10	miR-346	L10	miR-346	O10	miR-93-3p	P10	miR-93-3p
C11	miR-142-5p	D11	miR-142-5p	G11	miR-20a-5p	H11	miR-20a-5p	K11	miR-34c-5p	L11	miR-34c-5p	O11	miR-96-5p	P11	miR-96-5p
C12	miR-143-3p	D12	miR-143-3p	G12	miR-20b-5p	H12	miR-20b-5p	K12	miR-361-5p	L12	miR-361-5p	O12	miR-96-3p	P12	miR-96-3p
C13	miR-144-3p	D13	miR-144-3p	G13	miR-212-3p	H13	miR-212-3p	K13	miR-363-3p	L13	miR-363-3p	O13	miR-98-5p	P13	miR-98-5p
C14	miR-145-5p	D14	miR-145-5p	G14	miR-215	H14	miR-215	K14	miR-369-3p	L14	miR-369-3p	O14	miR-99a-5p	P14	miR-99a-5p
C15	miR-146a-5p	D15	miR-146a-5p	G15	miR-217	H15	miR-217	K15	miR-372	L15	miR-372	O15	miR-99a-3p	P15	miR-99a-3p
C16	miR-146b-5p	D16	miR-146b-5p	G16	miR-218-5p	H16	miR-218-5p	K16	miR-373-3p	L16	miR-373-3p	O16	miR-99b-5p	P16	miR-99b-5p
C17	miR-147a	D17	miR-147a	G17	miR-22-3p	H17	miR-22-3p	K17	miR-374a-5p	L17	miR-374a-5p	O17	C. e miR-39	P17	C. e miR-39
C18	miR-148a-3p	D18	miR-148a-3p	G18	miR-221-3p	H18	miR-221-3p	K18	miR-375	L18	miR-375	O18	C. e miR-39	P18	C. e miR-39
C19	miR-148b-3p	D19	miR-148b-3p	G19	miR-222-3p	H19	miR-222-3p	K19	miR-379-5p	L19	miR-379-5p	O19	RNU6-2-F	P19	RNU6-2-F
C20	miR-149-5p	D20	miR-149-5p	G20	miR-223-3p	H20	miR-223-3p	K20	miR-382-5p	L20	miR-382-5p	O20	RNU6-2-F	P20	RNU6-2-F
C21	miR-150-5p	D21	miR-150-5p	G21	miR-224-5p	H21	miR-224-5p	K21	miR-383	L21	miR-383	O21	SNORD43-F	P21	SNORD43-F
C22	miR-151a-3p	D22	miR-151a-3p	G22	miR-23a-3p	H22	miR-23a-3p	K22	miR-409-3p	L22	miR-409-3p	O22	SNORD43-F	P22	SNORD43-F
C23	miR-152	D23	miR-152	G23	miR-23b-3p	H23	miR-23b-3p	K23	miR-422a	L23	miR-422a	O23	SNORD95-F	P23	SNORD95-F
C24	miR-155-5p	D24	miR-155-5p	G24	miR-24-3p	H24	miR-24-3p	K24	miR-423-3p	L24	miR-423-3p	O24	SNORD95-F	P24	SNORD95-F

Protocol

Workflow Overview

A miRStar Human Cancer Focus miRNA PCR Array experiment consists of several major steps in a workflow shown in Figure 2.

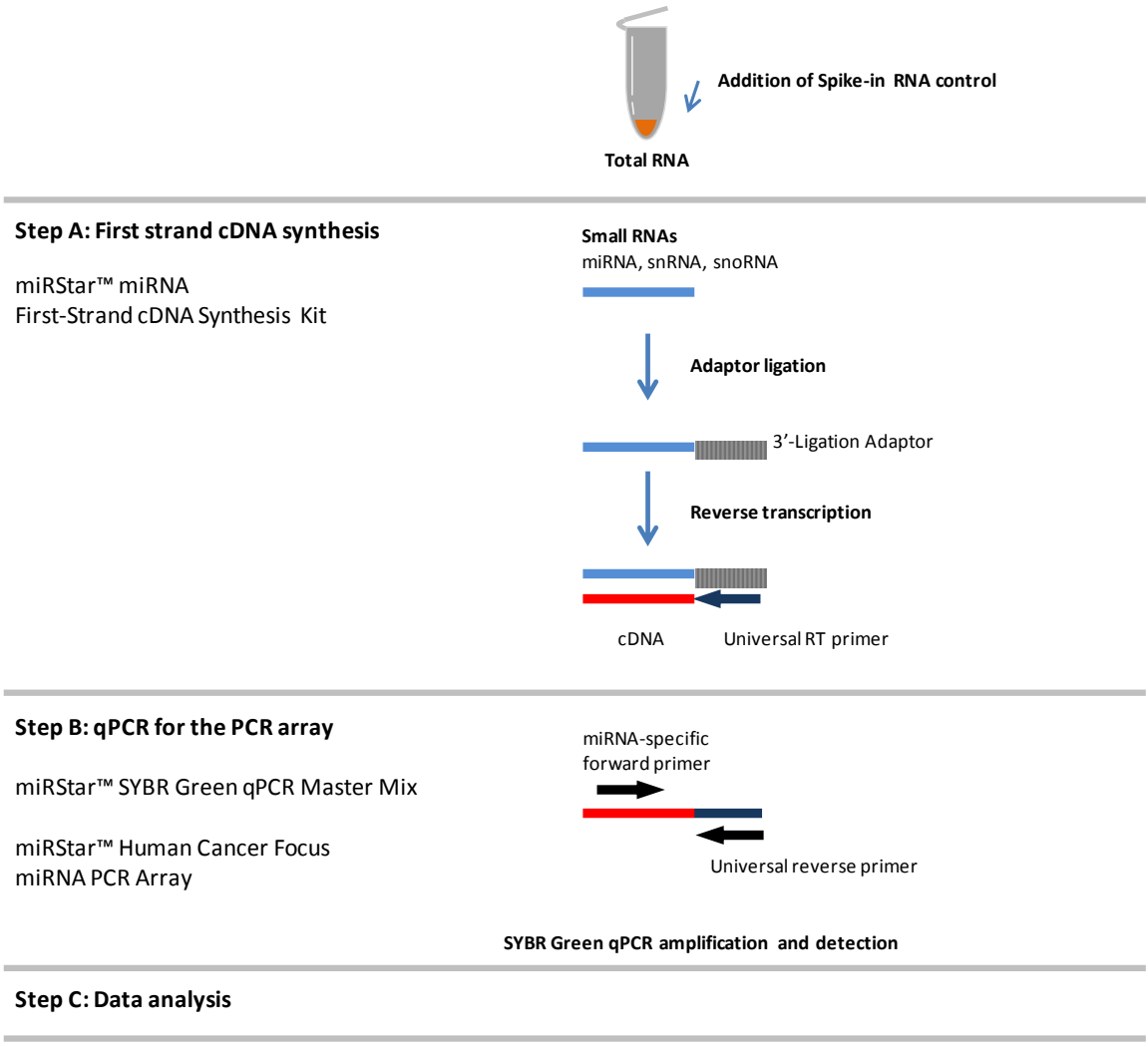

Figure 2. Workflow overview of miRStar™ Human Cancer Focus miRNA PCR Array experiment.

Step A. First-strand cDNA synthesis

Total RNA samples should be extracted by a method that can recover small RNA fraction, for example, TRIzol® Reagent method.

High quality cDNA synthesis is vital for the following qPCR performance. We highly recommend Arraystar miRNA First-Strand cDNA Synthesis Kit (Cat# AS-MR-004), which is fully compatible with and is specifically optimized for miRStar Human Cancer Focus miRNA PCR Array. Please refer to the Instruction Manual of the Kit for its use.

1. Dilute the 3' Ligation Adapter from the Kit with RNase-free water. The dilution factor is 1/10 for 10 - 500 ng or 1/3 for 0.5 - 2 μg of the starting total RNA. Use the same amount of total RNA for each sample in the experiment.
2. Set up the adaptor ligation reaction in a 200 μL PCR tube using the following components for each sample:

4.5 μL	Total RNA in nuclease-free water
1.0 μL	diluted 3' Ligation Adapter
1.0 μL	RNA Spike-in
6.5 μL	total volume

3. Incubate in a thermal cycler at 70°C for 2min; chill on ice immediately.
4. Add the following reagents and mix well. The final volume will be 10 μL.

2.0 μL	5×Ligase Reaction Mix
1.0 μL	RNA ligase
0.5 μL	RNase Inhibitor
10.0 μL	final volume

5. Incubate at 22°C for 60 min; 72°C for 2 min; and on ice for 2 min.

6. For reverse transcription, add 1 µL Universal RT Primer Mix, mix gently.
7. Incubate at 65°C for 2 min; place on ice for at least 2 min.
8. Prepare Reverse Transcription Master Mix and add 10 µL to each sample above.

8.5 µL	RT Reaction Master Mix
0.5 µL	RNase Inhibitor
1.0 µL	MMLV Reverse Transcriptase
10.0 µL	total volume per sample

9. Incubate at 42°C for 60 min; inactivate the reaction at 85°C for 5 min.

Step B. Perform qPCR for the PCR array

1. Dilute the cDNA in nuclease free water. If Arraystar miRNA First-Strand cDNA Synthesis Kit (Cat# AS-MR-004) is used for the cDNA synthesis with 10 ng - 2.0 µg total RNA sample as the starting material, dilute the cDNA product 1/80 in water. The diluted material is used as the qPCR template.
2. Use Arraystar SYBR Green Real-Time Quantitative PCR Master Mix to prepare qPCR Master Mix for each sample per qPCR well. There are total 384 reactions in a 384-well qPCR array plate, 192 wells for miRNA and 192 wells for mRNA (Figure 1). Add some extra reactions as needed by the liquid handling operation. Multiply this number with the individual amounts of the components in the table below and prepare a qPCR Mix.

5.0 µL	SYBR Green Master Mix

1.5 µL	Diluted cDNA template
3.5 µL	ddH_2O
10.0 µL	total volume per well

3. Dispense 10 µl of the Mix uniformly to each well on the qPCR array plate.
4. Run the qPCR using the following program. Consult the instructions for the instrument for details.

Cycles	Temperature	Time
1	95 ℃	10 minutes
40	95 ℃	10 seconds
	55-65 ℃	1 minute
Melting curve analysis		

Step C. Data analysis

1. Calculate the Δ*Ct* for each miRNA:

$$\Delta Ct_{\text{miRNA}} = Ct_{\text{miRNA}} - \text{average}\,(Ct_{\text{control}})$$

Where Ct_{control} are the values taken from one or more duplicates of the miRNA references (**CT**).

If no particular reference gene(s) are designated as the quantification reference, all the CTs can be averaged and used in the above formula, but only if the difference between the averaged values is less than 1 cycle when comparing the two groups.

2. Calculate the $\Delta\Delta Ct$ between two samples or groups for a gene:

$$\Delta\Delta Ct = \Delta Ct_{\text{sample2}} - \Delta Ct_{\text{sample1}}, \text{ or}$$

$$\Delta\Delta Ct = \Delta Ct_{\text{group2}} - \Delta Ct_{\text{group1}}$$

Where sample1 or group1 is the control and sample2 or group2 is the experimental.

3. Calculate the fold change from group 1 to group 2 for a gene as:

$$\textit{fold change} = 2^{-\Delta\Delta Ct}$$

OPTIONAL: If the fold-change is greater than 1, the result may be reported as a fold up-regulation. If the fold-change is less than 1, the negative reciprocal may be reported as a fold down-regulation.

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