



miRCURY LNA™ microRNA Array

Extended Spike-in microRNA Kit

Instruction manual
for product # 208041

Literature citations

Please refer to miRCURY LNA™ microRNA Array Extended Spike-in microRNA Kit when describing a procedure for publication using this product.

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The miRCURY LNA™ Array Power labeling kit contains the fluorescent dyes Oyster® 656 and Oyster® 556. Oyster® is a registered trademark of Denovo Biolabels GmbH.

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Product summary

4

Content

miRCURY LNA™ microRNA Array, Extended Spike-in microRNA Kit

2 vials each containing 52 synthetic unlabeled microRNAs, dried-down.

Each vial is sufficient for minimum 24 rxns.

1 vial containing 500 µL nuclease-free water.

Additional required material

miRCURY™ RNA Isolation Kits

Get high quality total RNA suitable for miRCURY LNA™ microRNA Array analysis in as little as 20 minutes. Protocols are available for a large number of sample types and organisms.

miRCURY LNA™ microRNA Array, microarray kits

Pre-spotted microarrays for determination of microRNA expression patterns. The arrays contains capture probes for the 52 Spike-in microRNAs.

miRCURY LNA™ microRNA Array, ready to spot probe set

Capture probe set for spotting of microarrays. The set contains capture probes for all annotated microRNAs in miRBase 11 as well as for the 52 Spike-in microRNAs.

miRCURY LNA™ microRNA Power labeling kit

Fluorescent labeling of microRNAs from total RNA samples ready for hybridization to arrays (cat# 208030-A, 208031-A, 208032-A).

For manual hybridization

Microarray Hybridization Chamber - SureHyb (Agilent product # G2534A)

Glass Coplin staining jar/dish or equivalent for manual hybridization.



Product description

The Extended Spike-in microRNA Kit contains 52 different synthetic unlabeled plant microRNAs in a range of concentrations. All 52 synthetic microRNAs are 5' phosphorylated just like endogenous microRNAs. The microRNAs can be spiked into an RNA sample prior to labeling and the synthetic Spike-in kit will hybridize to corresponding capture probes on the miRCURY LNA™ microRNA Array. The 52 microRNAs in the Extended Spike-in microRNA Kit resemble microRNAs from the plant *arabidopsis thaliana* and have been tested not to cross-react with endogenous microRNAs from human, mouse or rat. The Extended Spike-in microRNA Kit is supplied with different concentrations of synthetic Spike-in microRNAs aimed at spanning the whole intensity range of microRNAs in most tissue samples. The corresponding capture probes have been printed in 4 replicates each on the arrays.

Prior to use, the Extended Spike-in microRNAs must be dissolved in 15 µL of nuclease-free water. Leave the solution on ice for 30 minutes to dissolve. Vortex and then spin to collect tube contents. Exiqon recommends to aliquot the dissolved Spike-in microRNAs to avoid repeated freeze/thawing. For long-term storage, keep the vial at -80°C.

Note

Use 0.5 µL of the Extended Spike-in microRNAs in each labeling reaction. For detailed procedure, please see the instruction manual of the miRCURY LNA™ microRNA Power labeling kit.

See tip
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The Extended Spike-in microRNA Kit can be used together with or instead of the regular microRNA Spike-in kit (cat#204080) that is supplied with the miRCURY LNA™ microRNA Array microarray kit. If they are to be used together, we recommend that each vial of Spike-in mix (both extended and regular) is dissolved in 15 µL RNase free water. 0.5 µL of each mix is then added to the CIP treatment prior to labeling.



The Power Labeling kit manual (Table 2) contains information about volumes for the CIP treatment mix. This table can be modified in 2 different ways depending on if both Spike-in kits are used or only the Extended Spike-in microRNA Kit:

Table 1

	Volume (μl) if both Spike-in kits are used	Volume (μl) if only Extended Spike-in kit is used
Total RNA	2	2.5
Spike-in microRNA kit	0.5	-
Extended Spike-in microRNA Kit	0.5	0.5
CIP buffer	0.5	0.5
CIP enzyme	0.5	0.5

Table 1. Volumes to use for the CIP treatment prior to labeling. These recommended volumes replace the Power Labeling kit manual Table 2.

When the Extended Spike-in microRNA Kit is added to labeling reactions before an array hybridization, the signals from the Spike-in capture probes can be used

- as a control of the labeling reaction and hybridization
- as a help in deciding scanner settings between channels
- as a control of the data normalization procedures
- to estimate the variance of replicated measurements within arrays
- to assess technical variability between different parts of the array and between different arrays in an experiment

The table shows the annotations of the Extended Spike-in microRNA capture probes available in the GAL-file for miRCURY LNA™ microRNA Arrays and in the microplate layout file for the miRCURY LNA™ microRNA Arrays ready-to-spot probe set. The files are located at www.exiqon.com/miRCURY/array

Table 2

Probe ID	Name
1100	extended_spike_control_1
13186	extended_spike_control_2
13367	extended_spike_control_3
13371	extended_spike_control_4
13388	extended_spike_control_5
13389	extended_spike_control_6
13393	extended_spike_control_7
13417	extended_spike_control_8
13421	extended_spike_control_9
13430	extended_spike_control_10
24127	extended_spike_control_11
24136	extended_spike_control_12
24163	extended_spike_control_13
24199	extended_spike_control_14
24217	extended_spike_control_15
24226	extended_spike_control_16
25557	extended_spike_control_17
25593	extended_spike_control_18
25611	extended_spike_control_19
25728	extended_spike_control_20
26160	extended_spike_control_21
27291	extended_spike_control_22
27318	extended_spike_control_23
27350	extended_spike_control_24
27676	extended_spike_control_25
27821	extended_spike_control_26
27833	extended_spike_control_27
27953	extended_spike_control_28
27968	extended_spike_control_29
28038	extended_spike_control_30
28098	extended_spike_control_31
28393	extended_spike_control_32
28444	extended_spike_control_33
28488	extended_spike_control_34
28568	extended_spike_control_35
28581	extended_spike_control_36
28684	extended_spike_control_37
28876	extended_spike_control_38
28929	extended_spike_control_39
29001	extended_spike_control_40
29056	extended_spike_control_41
29138	extended_spike_control_42
29146	extended_spike_control_43
29544	extended_spike_control_44
29564	extended_spike_control_45
29837	extended_spike_control_46
30147	extended_spike_control_47
30207	extended_spike_control_48
30293	extended_spike_control_49
30747	extended_spike_control_50
30756	extended_spike_control_51
32812	extended_spike_control_52

Table 2. The Extended Spike-in microRNA control capture probes and their probe ID's.



Guidelines for the Extended Spike-in microRNA signal distribution

The figure below shows the distribution of the 10 regular and 52 Extended Spike-in microRNAs spiked into 250 ng of total RNA from human lung samples. The concentration of the various Spike-in microRNAs are optimized so the signal intensities of these Extended Spike-in microRNAs are in the dynamic range of naturally expressed microRNAs in most tissues.

Note

The position of signals from the Spike-in microRNA kit compared to signals from microRNAs will depend upon the microRNA expression level in the sample.

Figure 1

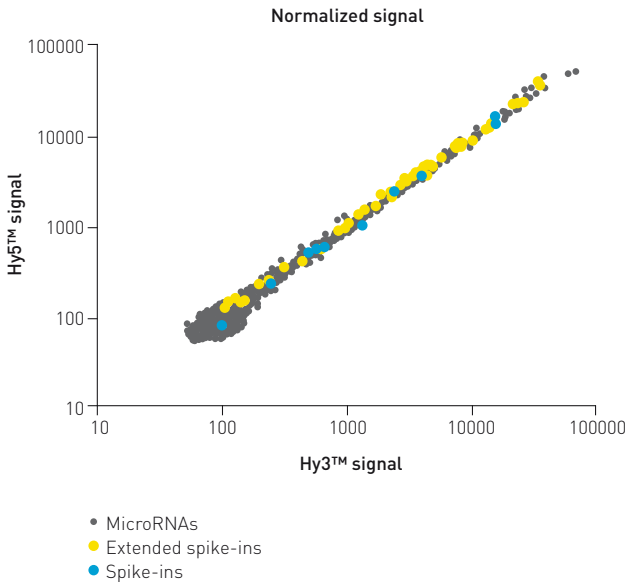


Figure 1. 0.5 μ L of the Extended Spike-in microRNAs were spiked into a sample of 250 ng total RNA from human lung labeled with Hy3™. Another, 0.5 μ L of Extended Spike-in microRNAs were spiked into 250 ng total RNA from human lung and labeled with Hy5™. Labeling was performed using the miRCURY LNA™ microRNA Power labeling kit. Hybridization was performed using a Tecan HS4800 hybridization station.

Storage

Dissolve each vial of Extended Spike-in microRNAs in 15 μl of nuclease-free water (supplied) upon receipt. Leave the solution on ice for 30 minutes to dissolve. Vortex and then spin to collect tube contents. Store the dissolved Spike-in microRNAs at -20°C until use and avoid repeated cycles of freeze/thawing.

Exiqon recommends to aliquot the dissolved Spike-in microRNAs into smaller volumes to avoid repeated freeze/thawing. For long-term storage, keep the vial at -80°C .



Tips and Trouble shooting

10 **Tip 1** Alternatively, the Extended Spike-in microRNA Kit can be used as a hybridization control

By labeling the Spike-ins separately from the samples and then adding them just prior to hybridization. We then suggest to dissolve each vial of Extended Spike-in microRNA mix in 3 μl water and mix Spike-in from the two vials. 3 μl Extended Spike-in microRNA mix is then labeled in Hy3 and 3 μl in Hy5 in standard labeling reactions. After labeling, mix 12.5 μl of each labeling reaction with 25 μl hybridization buffer. Use 1 μl of this mix together with each labeled RNA sample.



Related products



Exiqon offers a tool kit enabling new discoveries concerning the expression, function, and spatial distribution of microRNAs:

miRCURY™ RNA Isolation Kits

Get high quality total RNA suitable for miRCURY LNA™ microRNA Array analysis in as little as 20 minutes. Protocols are available for a large number of sample types and organisms. For more information, visit www.exiqon.com

miRCURY LNA™ microRNA Power Labeling kit

For fluorescent labeling of microRNAs from total RNA samples ready for array hybridization (product # 208030-A, 208031-A, 208032-A).

miRCURY LNA™ microRNA Array, microarray kit

Pre-printed miRCURY LNA™ microRNA Array microarray slides, available in pack sizes of 3, 6 and 24 (product # 208200-A, 208201-A, 208202-A).

miRCURY LNA™ microRNA Array, ready-to-spot probe set

Ready-to-spot oligo set for direct printing of arrays, or coupling in bead-based applications (product # 208210-A).

miRCURY LNA™ microRNA Array, Hybridization buffer

5 mL hybridization buffer optimal for microRNA hybridization to the miRCURY LNA™ microRNA Arrays (product # 208022).

miRCURY LNA™ microRNA Array, Wash buffer kit

125 mL salt buffer and 15 mL detergent optimal for wash of miRCURY LNA™ microRNA Arrays. (product # 208021).



miRCURY LNA™ microRNA Detection

For *in situ* hybridization and Northern blotting of all annotated microRNAs.

miRCURY LNA™ microRNA Knockdown

MicroRNA knockdown probes: Determine or confirm microRNA function.

miRCURY LNA™ microRNA Real-time PCR

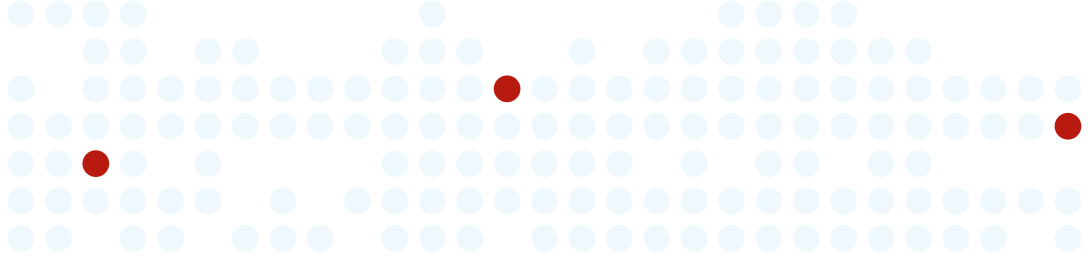
Quickly and accurately determine microRNA expression using real-time PCR system.



References

- The microRNA Registry
Griffiths-Jones S. *Nucleic Acids Research*, 2004, 32, Database Issue, D109-11
- miRBase, Wellcome Trust Sanger Institute. <http://microrna.sanger.ac.uk>
- www.exiqon.com/miRCURY/array





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