miRCURY LNA™ microRNA ISH Optimization Kit (FFPE)

microRNA *in situ* hybridization kit for FFPE samples. Optimize the procedure for your samples with the included DIG-labeled LNA[™] probes.

At a glance

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- The shortcut to successful microRNA ISH few experimental steps and a minimum of optimization
- Fast and easy one-day microRNA ISH protocol
- Superior sensitivity and specificity essential reagents and double DIG-labeled LNA™ probes for optimal ISH analysis
- Very robust can be used for both high throughput and individual microRNA localization studies
- Highly flexible no advanced instruments needed
- Validated in a wide range of tissues ideal for use with clinical and experimental FFPE samples

The easiest way to get started with microRNA ISH

A miRCURY LNA[™] microRNA ISH Optimization Kit (FFPE) is the ideal option for getting started with or optimizing microRNA *in situ* hybridization (ISH) experiments on formalin-fixed paraffin embedded (FFPE) tissue samples.

Based on the popular and highly sensitive double (5' and 3') DIG-labeled miRCURY LNA™ microRNA Detection Probes, the kits provide the sensitivity and specificity needed to perform successful microRNA ISH analysis (Figure 1). All the kits come with reagents, including a non-toxic, formamide-free ISH buffer, specifically adapted for use with LNA™ probes in FFPE tissue sections.

Use the included probes to optimize the procedure for your samples. Then use double DIG-labeled miRCURY LNA™ microRNA Detection Probes to detect your microRNAs of interest.

The accompanying instruction manual carefully explains each step of the ISH procedure and provides tips and recommendations for a successful experiment. Furthermore, it includes a thoroughly validated one-day protocol for fast and trouble-free ISH analysis.

Flexible and robust

The kits can be used for a large number of applications including cellular and sub-cellular microRNA localization studies and determination of spatial microRNA expression.

Exiqon's scientists have developed a simplified protocol which eliminates several of the steps normally associated with ISH, such as pre-hybridization, post-fixation and acetylation, thus making the protocol very robust and easy to optimize. Furthermore, the procedure is completely formamide-free and non-radioactive, which minimizes the exposure to harmful chemicals. Taken together, the flexibility of the kits makes them ideal for use in both clinical and research laboratories and for use in both automated and manual set-ups.

A solution for every sample

Seven different miRCURY LNA™ microRNA ISH Optimization Kits are available. Each kit comes with positive and negative control probes, hybridization buffer and Proteinase K. A unique tissuespecific miRCURY LNA™ microRNA Detection Probe is included in each kit. These probes can be used as positive control probes, as part of the initial optimization procedure.

The unique microRNA LNA[™] probes have been validated in a variety of tissues and cell types (see table on back and figures 2-4).

Figure 1. Overview of the procedure. First, the tissue is "opened" using Proteinase K. In the hybridization step, the double DIG-labeled LNA™ probe binds specifically to its target microRNA. Alkaline phosphatase (AP)-conjugated anti-DIG antibodies are then added. This step is followed by NBT-BCIP development and optional counter-staining with Nuclear Red.







Selecting the appropriate miRCURY LNA™ microRNA ISH Optimization Kit.

The table indicates the tissue(s) and cell types in which each of the kits has been validated.

		Kit 2						
Brain				1				
Eye				1	1			
Muscle	1				1			
Lung					1	1		
Kidney					1			
Liver			1		1			
Colon					1	1		1
Cervix							1	
Heart	1				1	1		
Mammary Gland					1		1	
Lung cancer		1			1	1	1	
Colorectal cancer		1			1	1		
Breast cancer		1			1	1	1	
Kidney cancer		1			1	1		
Cervix cancer		1			1	1	1	
Testis cancer					1	1		
Esophagus cancer								1
Cell entity	myocyte	varies	hepa- tocyte	neuron	endo- thelial	smooth muscle	basel cells	granu- locyte

Product coverage

- Unique microRNA LNA™ probe (double DIG-labeled, kit specific)
- Scrambled LNA[™] probe (double DIG-labeled, negative control)
- U6 LNA™ probe (5' DIG-labeled, positive control)
- Hybridization buffer (2x, formamide-free)
- Proteinase K (12 mg, lyophilized)

Selected publications

Jørgensen *et al.* Methods 2010, 52: 375-81 Nielsen *et al.* Clin. Exp. Metastasis 2011, 28: 27-38 Hosoda *et al.* Circulation 2011, 123: 1287-96

For updated product information, please visit **www.exiqon.com/mirna-ish-kit**

Ordering information

microRNA ISH Optimization Kit	Product no.
Kit 1 (miR-1) Includes controls and buffer	90001
Kit 2 (miR-21) Includes controls and buffer	90002
Kit 3 (miR-122) Includes controls and buffer	90003
Kit 4 (miR-124) Includes controls and buffer	90004
Kit 5 (miR-126) Includes controls and buffer	90005
Kit 7 (miR-145) Includes controls and buffer	90007
Kit 8 (miR-205) Includes controls and buffer	90008
Kit 9 (miR-223) Includes controls and buffer	90009
Other reagents	Product no.
microRNA ISH Buffer 25ml (1000 slides)	90001
microRNA ISH Buffer and Controls kit	90010

Did you know?

microRNA ISH kits can also be used for fresh frozen samples. Go to **www.exiqon.com/ish** for more information.



Figure 2. miR-126 detection in colon wall. Kit 5 can be used to detect microRNAs in inflamed colon FFPE tissue. Here, it was used to detect miR-126. Staining was performed with NBT-BCIP (blue). Sections were counterstained with nuclear red.



Figure 3. miR-145 detection in human colon. Kit 7 can be used to detect microRNAs in colon FFPE tissue. Here, miR-145 is detected in a human colon wall with underlying muscle layers. Staining was performed with NBT-BCIP (blue). Sections were counterstained with nuclear red.



Figure 4. miR-205 detection in human breast carcinoma. Kit 8 can be used for detection of microRNAs in breast cancer FFPE tissue. Here, it was used to detect miR-205. Staining was performed with NBT-BCIP (blue). Sections were counterstained with nuclear red.

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