Guidelines for *in situ* hybridization on TSA™ Plus Fluorescence systems using the miRCURY LNA™ ISH Optimization Kits

The DIG labeled LNA™ probes used in the miRCURY LNA™ ISH Optimization kit can be detected by using alternative methods for DIG detection such as the TSA™ based systems. The use of the TSA™ based systems can be recommendable for detection of low copy targets in order to increase the signal.

These guidelines are a supplement to the miRCURY LNA™ microRNA ISH Optimization Kit (FFPE) manual v2.0. The manual can be downloaded from www.exiqon.com/mirna-ish-kit.
Modification of the manual is according to Perkin Elmer manual TSA™ PLUS Fluorescence Systems. The manual can be downloaded from www.perkinelmer.com/Catalog/Product/ID/NEL741001KT.

Additional required reagents, not supplied:
- TSA™ Fluorescein System (NEL741001KT/NEL741B001KT, Perkin Elmer).
- Anti-DIG-POD (11207733910, Roche Applied Science).
- SlowFade® Gold antifade reagent with DAPI (S36938, Life Technologies).
- Hydrogen peroxide solution, 3% (e.g. 88597, Sigma-Aldrich).
- DMSO (HPLC grade).

Before starting

Before starting the experiment, please refer to the miRCURY LNA™ microRNA ISH Optimization Kit (FFPE) manual v 2.0 pages 12-17 for general recommendations and precautions for RNA work.

Prepare the following additional reagents:

Fluorophore Tyramide solutions:
Stock solution: add 150 µl DMSO to the vial.
Working solution (prepare just before use): 1:50 with 1x Plus amplification diluent.

microRNA ISH protocol

The One-day microRNA ISH protocol is described in detail in the miRCURY LNA™ microRNA ISH Optimization Kit (FFPE) manual pages 18-22. When using TSA™ Plus Fluorescence systems, some steps in the protocol should be replaced.

Step 1-2	Follow steps 1-2 in the protocol as described (pages 18-19).

Step 3 in the protocol is replaced by the following steps:

Step 3a Wash in PBS	Place slides into a slide rack inside a jar with PBS, wash twice in PBS.
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Step 3b Block peroxidase 2x3 min	Block endogenous peroxidase activity with 3% H_2O_2 for 2x3 min at room temperature (RT).
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Step 3c Wash in PBS	Place slides into a slide rack inside a jar with PBS, wash twice in PBS.

Step 4-9	Continue to step 4-9 (pages 19-21) in the protocol as described.

Step 10 in the protocol is replaced by the following step:

Step 10 Apply anti-DIG-POD antibody	Remove blocking solution and incubate slides with anti-DIG-POD 1:400 for 60min at RT. **NOTE:** The blocking dilutant solution described in table 3, page 15 is used, but only 1:400 dilution of the antibody!

Step 11-13 in the protocol is replaced by the following steps:

Step 11 3x3 min wash in PBS	Wash the slides 3x3 min with PBS.
Step 12 Incubate with TSA-plus FITC 2x5 min at RT	Apply TSA-plus FITC substrate to the sections and incubate slides 2x5 min at RT.
Step 13 3x5 min wash in PBS	Wash the slides 3x5 min in PBS buffer to stop the reaction.
Step 14-17	These steps in the protocol is omitted when using the TSA™ Plus Fluorescein System.
Step 18 Mount slides	Mount slides directly with SlowFade® Gold antifade reagent with DAPI. Avoid air-drying sections at this step.
Step 19	Continue to step 19 (page 22) in the protocol as described.

For Tips & Troubleshooting see the miRCURY LNA™ microRNA ISH Optimization Kit (FFPE) manual v2.0

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Contact information
Outside North America
Phone: +45 45 65 09 29 · Fax: +45 45 66 18 88

North America
Phone: +1 781 376 4150 · Fax: +1 781 376 4152
www.exiqon.com

920499 - 07/2012