

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.



Step 3b

Block peroxidase 2x3 min

Block endogenous peroxidase activity with 3% H₂O₂ for 2x3 min at room temperature (RT).



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 Remove blocking solution and incubate slides with anti-DIG-POD 1:400 for 60min
Apply anti-DIG-POD antibody 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 Wash the slides 3x3 min with PBS.
3x3 min wash in PBS

Step 12 Apply TSA-plus FITC substrate to the sections and incubate slides 2x5 min at RT.
Incubate with TSA-plus FITC 2x5 min at RT

Step 13 Wash the slides 3x5 min in PBS buffer to stop the reaction.
3x5 min wash in PBS

Step 14-17 These steps in the protocol is omitted when using the TSA™ Plus Fluorescein System.

Step 18 Mount slides directly with SlowFade® Gold antifade reagent with DAPI.
Mount slides 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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