Guidelines for *in situ* hybridization on TSA™ Plus DNP AP 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 are according to Perkin Elmer manual Renaissance® - TSA™ PLUS DNP (HRP or AP) Systems. The manual can be downloaded from www.perkinelmer.com/Catalog/Product/ID/NEL746A001KT.

Additional required reagents, not supplied:
- TSA™ Plus DNP AP (NEL746B001KT/NEL746A001KT, Perkin Elmer).
- Anti-DIG-POD (11207733910, Roche Applied Science)
- 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:

DNP amplification reagent:
Stock solution: add 150 µl DMSO (HPLC grade) to the vial.
Working solution (prepare just before use): dilute 1:50 with 1xPlus 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 v 2.0 pages 18-22. When using TSA™ Plus DNP AP systems, some steps in the protocol should be replaced.

Step 1-9	Follow steps 1-9 in the protocol as described (pages 18-21).

Step 10-12 in the protocol is replaced by the following steps:

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 11a 3x5 min wash in PBS-T	Wash slides 3x5 min in PBS-T buffer at RT.

Step 11b Incubate with DNP reagent for 2x5 min	Incubate slides with DNP amplification reagent (working solution) for 2x5 min at RT. **NOTE:** The amplification solution is prepared as described above.
Step 11c 3x5 min wash in PBS-T	Wash slides 3x5 min in PBS-T buffer at RT.
Step 11d Incubate with anti-DNP-AP for 2x15 min	Incubate slides with anti-DNP-AP 1:100 for 2x15 min at RT. Use the the blocking dilutant solution described in table 3, page 15.
Step 11e 3x5 min wash in PBS-T	Wash slides 3x5 min in PBS-T buffer at RT.
Step 12 Incubate with AP substrate for 10-60 min at RT	Incubate slides with AP substrate for 10 minutes at RT in the dark. Examine slides for signal strength. If a darker signal is desired, incubate slides an additional 10-30 min, max 60 min.
Step 13-19	Follow step 13-19 as described (p. 21-22).

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

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