

## 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](http://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](http://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.

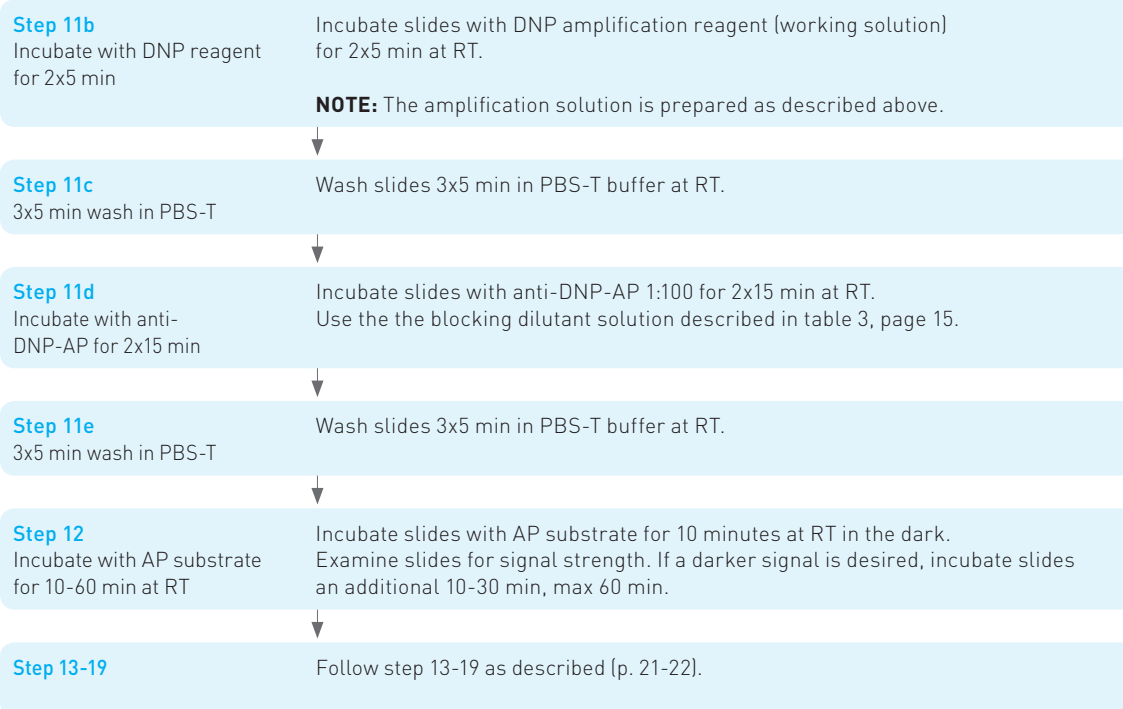
**NOTE:** The blocking dilutant solution described in table 3, page 15 is used, but only 1:400 dilution of the antibody!

#### Step 11a

3x5 min wash in PBS-T

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





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

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