

# ISH-IHC PROTOCOL FOR CHROMOGENIC DETECTION ON FORMALIN FIXED PARAFFIN EMBEDDED (FFPE) TISSUE

## Materials

### ISH Reagents from ACD

RNAscope® 2.5 HD Reagent Kit-RED (Cat No. 322350) which includes:

HD Detection Kit (RED) (Cat# 322360)

Wash Buffer (Cat No. 310091)

Target Retrieval Reagent (Cat No. 322000)

Protease and Peroxidase Blocking Reagent (Cat No. 322330)

### IHC Reagents

Normal Serum Blocking reagent

Green HRP Chromogen

IHC Validated Primary Antibody

HRP- Polymer Conjugated Secondary Antibody

Gill's Hematoxylin I (50%)

VectaMount™ Mounting Medium (or equivalent)

### ISH Staining with ACD's RNAscope Assay

See RNAscope® 2.5 HD Reagent Kit-RED Assay User Manual. The tissue-specific [pretreatment conditions for FFPE tissues](#) outlined below for Target Retrieval step are only recommendations. It may be necessary to optimize protease digestion for the IHC target of choice. *Always follow user manual protocols that correspond to their appropriate kits purchased from ACD.*

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### IHC Staining

IHC staining should be performed immediately following ISH chromogenic detection.

**Note:** PBS buffer decolorizes green chromogen. When using green chromogen, use only wash buffer provided by ACD for all washing steps. All steps are done at room temperature unless specified differently.

1. Wash slides twice for 2 minutes in wash buffer.
2. Incubate tissue sections for 15 minutes in 200 $\mu$ L of Normal Serum Blocking Reagent. Tap solution off tissue sections- do not rinse slides.
3. Using experimentally predetermined optimal conditions, incubate slides in 200 $\mu$ L of primary antibody solution.
4. Wash slides twice for 2 minutes in wash buffer.
5. Apply 200 $\mu$ L of HRP polymer-conjugated secondary antibody and incubate for 30 minutes.
6. Wash slides three times for 2 minutes each in wash buffer.
7. While your slides are washing, make a 1:50 working solution of a chromogen by mixing Green B (1  $\mu$ L) and Green A components (49  $\mu$ L).
8. Apply 200  $\mu$ L of green HRP solution to slides and incubate for 10-15 minutes.
9. Quickly tap green HRP solution off slides and *briefly* rinse with distilled water.  
**Note:** Green substrate can lose its color by extended exposure to water.
10. Counterstain with 50% Hematoxylin I for 30 seconds. Gently rinse slides *briefly* with distilled water and place on bench top to dry or use oven to speed up drying procedure.
11. Once dry, use a small amount of media to mount tissues.