

II. PROTOCOL

A. Summary Protocol for TSA-Direct for In Situ Hybridization

<i>Blocking Step and Incorporation of HRP</i>	Final post hybridization wash.
	<u>↓</u>
	Wash slides 3X for 5 min. in TNT buffer @ R.T.
	<u>↓</u>
	Block slides for 30 min. in TNB buffer @ R.T.
	<u>↓</u>
	OR
	<u>↓</u>
Slides react with SA-HRP for 30 min. @ R.T.	Slides react with labeled antibody for 30 min. @ R.T.
<u>↓</u>	<u>↓</u>

Wash slides 3X for 5 min. in TNT buffer @ R.T.

**TSA
Amplification**



Incubate in Fluorophore Tyramide for 3 to 10 min. @ R.T.



Wash slides 3x for 5 min. in TNT buffer @ R.T.



FLUORESCENCE

Visualization

Add appropriate counterstain and mount for fluorescence microscopy

CHROMOGENIC

for NEL731, 731A only

Incubate slides in Antifluorescein-HRP or Antifluorescein-AP for 30 min. @ R.T.



Wash slides 3x for 5 min. in TNT buffer @ R.T.



Add appropriate chromogenic substrate, counterstain and mount for microscopy

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