START-UP GUIDE

TSA™ IHC / ICC / IF Optimization

Tyramide signal amplification (TSA) typically provides 2-3 logs of sensitivity enhancement over standard detection methods (for example, fluorophore-labeled secondary antibodies or enzymatic detection with a chromogenic substrate) while maintaining resolution and allowing reduced consumption of primary antibodies. Protocol optimization for TSA detection is critical for success with the technique.

Important information

- Horseradish peroxidase (HRP) is necessary to drive the TSA labeling reaction.
- If present, endogenous peroxidases should be quenched to prevent non-specific detection.
- If HRP concentration is too high, formation of TSA dimers will be favored over the labeling reaction, leading to reduced signal and higher background. For this reason, it is critical to optimize dilution of primary antibody and HRP reagent.
- Use of a blocking reagent is highly recommended. PerkinElmer offers a blocking reagent that is optimal for use with TSA (catalog number FP1012).
- TSA stock solution should be diluted 1:50 in amplification diluent. Problems with excess signal can be resolved by using more dilute antibody solutions for detection. This approach ensures the highest specificity in detection.
- First time TSA users should apply this to a proven IHC system, and include an unamplified control slide.

Suggested optimization procedure for first time use of TSA

TSA is ideal for detection of targets that have weak signals using standard detection methods. It enables use of much more dilute antibody solutions for improved specificity. If the signal is strong with standard detection methods, dilute the primary antibody until the signal begins to disappear and then proceed with TSA optimization in the same manner.

1. Primary antibody optimization	2. Introduction of HRP: Select an option	3. Detection
Slide 1: Dilution specified by current method or recommended by primary Ab manufacturer **Slide 2:** 5-fold dilution from slide 1 **Slide 3**: 5-fold dilution from slide 2 **Slide 4:** 5-fold dilution from slide 3 (further dilution may be needed) **Slide 5:** Negative control (no primary antibody)	Secondary antibody HRP conjugates Anti-mouse-HRP, (catalog number NEF822001EA) Anti-rabbit-HRP, (catalog number NEF812001EA) Recommended starting range of 1:500 to 1:2000. Further dilution may be needed SA-HRP reagent (catalog number NEL750001EA) 1:1250-1:2500 dilution Anti-fluorescein-HRP (catalog number NEF710001EA) 1:100-1:500 dilution HRP reagents from other manufacturers Begin with recommended range for slide applications. Further dilution may be needed.	TSA 1:50 in amplification diluent

For detailed information on TSA assay development, please refer to the product manual available online at **www.perkinelmer.com/tsa** or contact PerkinElmer technical support via e-mail at **global.techsupport@perkinelmer.com.**

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TSA Stock Solutions

Kit	Catalog Numbers	Solvent	Volume per tube
TSA Plus Biotin	NEL749A, NEL749B	DMSO	300 μL
TSA Plus DNP, 25-75 slides	NEL746B, NEL747B	DMSO	150 μL
TSA Plus DNP, 50-150 slides	NEL746A, NEL747A	DMSO	300 μL
TSA Plus Fluorescence Kits	Kits beginning with NEL741-745	DMSO	150 μL
TSA Plus Multi-Fluor Kits	Kits beginning with NEL752-756	DMSO	150 μL
TSA Plus Palette Kit	NEL760	DMSO	60 μL
TSA Biotin, 200-600 slides	NEL700	DMSO	1200 μL
TSA Biotin, 50-150 slides	NEL700A	DMSO	300 μL
TSA Fluorescence Kits, 100-300 slides	NEL701, NEL702, NEL703	DMSO	600 μL
TSA Fluorescein, 50-150 slides	NEL701A	DMSO	300 μL
TSA Cyanine 3 or 5, 50-150 slides	NEL704A, NEL705A	H_2O	300 μL

Dilute stock solutions 1:50 in amplification diluent to make working solution. DMSO should be molecular biology or HPLC grade.

Protocol for TSA IHC/ICC/IF

Standard IHC

Quench endogenous peroxidase activity (if needed)
Block slides for 30 min. in TNB buffer at room temperature
Incubate slides in primary antibody for 30-60 minutes at room temperature
Wash slides 3X for 5 min. in TNT buffer at room temperature

Incorporation of HRP

Incubate slides in HRP labeled secondary antibody for 30 min. at room temperature

or

Incubate slides in biotinylated secondary antibody 30-60 min. at room temperature
Wash slides 3X for 5 min. in TNT buffer
Incubate slides in SA-HRP for 30 min.

Wash slides 3X for 5 min. in TNT buffer at room temperature

TSA Plus Amplification

Incubate in Amplification Reagent working solution for 3 to 10 min. at room temperature

Wash slides 3X for 5 min. in TNT buffer at room temperature

Visualization

Direct Fluorescence	Indirect Fluorescence	Chromogenic
	Incubate slides in fluorophore conjugate for 30 min.	Incubate slides in HRP or AP conjugate for 30 min.
Wash slides 3X for 5 min. in TNT buffer at room temperature	Wash slides 3X for 5 min. in TNT buffer at room temperature	Wash slides 3X for 5 min. in TNT buffer at room temperature
Counterstain and mount for fluorescence microscopy	Counterstain and mount for fluorescence microscopy	Add appropriate chromogen, counterstain and mount for microscopy

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