

Caution: For Laboratory Use. A research chemical for research purposes only.

## TSA Plus Fluorescence Kits

### Product Information

- Storage:** Store kits in the dark at 4°C.
- Stability:** The components in the kits are stable for a minimum of 3 months under proper storage conditions.
- Application:** TSA Plus Fluorescence kits are intended for high sensitivity detection in immunohistochemistry (IHC), immunocytochemistry (ICC) and *in situ* hybridization (ISH) experiments. Final detection may be fluorescent or chromogenic (with TSA Plus Fluorescein kits only).

### FOR RESEARCH USE ONLY.

### Safety Note

All reagents are classified as nonhazardous. However, it is strongly recommended to wear disposable gloves and safety glasses while working. Thorough washing of hands after handling is also recommended.

**Quality Control** We certify that QC results of these reagents meet our quality release criteria.

### What is TSA technology?

TSA<sup>®</sup> from PerkinElmer is a technology that improves fluorescent and chromogenic signals by up to 100-fold while allowing reduced consumption of primary antibodies or probes. TSA Plus is an optimized and enhanced version that is up to 20 times more sensitive than standard tyramide reagents.

TSA Plus Fluorescence Kits use horseradish peroxidase (HRP) to catalyze covalent deposition of fluorophores directly adjacent to the immobilized enzyme. The labeling reaction is quick (less than 10 minutes) and deposited labels can then be viewed directly using standard or confocal microscopy. TSA Plus Fluorescein may also be used in combination with anti-fluorescein enzyme conjugates and appropriate chromogenic substrates for brightfield microscopy.

The use of TSA reagents results in a significant increase in sensitivity over standard detection methods, while maintaining specificity and resolution. Moreover, TSA reagents allow drastically reduced consumption of primary antibody or probe.

TSA Plus Fluorescence kits are ideal for multiplexed IHC or ISH in combination. The Opal<sup>™</sup> protocol allows simultaneous measurement of 6 or more biomarkers in FFPE tissue. For more information, please see [www.perkinelmer.com/opal](http://www.perkinelmer.com/opal).

## Material Provided

	Format*	Catalog #	Kit Components
<b>TSA Plus Fluorescein</b>	50-150 slides	NEL741001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (15 mL)</li> <li>• Fluorescein Plus Amplification Reagent (2 tubes, add 150 µL DMSO / tube)</li> </ul>
	250-750 slides	NEL741B001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (5 x 15 mL)</li> <li>• Fluorescein Plus Amplification Reagent (10 tubes, add 150 µL DMSO / tube)</li> </ul>
	25-75 slides	NEL741E001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (7.5 mL)</li> <li>• Fluorescein Plus Amplification Reagent (1 tube, add 150 µL DMSO)</li> </ul>
<b>TSA Plus Tetramethylrhodamine</b>	50-150 slides	NEL742001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (15 mL)</li> <li>• TMR Plus Amplification Reagent (2 tubes, add 150 µL DMSO / tube)</li> </ul>
	250-750 slides	NEL742B001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (5 x 15 mL)</li> <li>• TMR Plus Amplification Reagent (10 tubes, add 150 µL DMSO / tube)</li> </ul>
<b>TSA Plus Cyanine 3</b>	50-150 slides	NEL744001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (15 mL)</li> <li>• Cyanine 3 Plus Amplification Reagent (2 tubes, add 150 µL DMSO / tube)</li> </ul>
	250-750 slides	NEL744B001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (5 x 15 mL)</li> <li>• Cyanine 3 Plus Amplification Reagent (10 tubes, add 150 µL DMSO / tube)</li> </ul>
	25-75 slides	NEL744E001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (7.5 mL)</li> <li>• Cyanine 3 Plus Amplification Reagent (1 tube, add 150 µL DMSO)</li> </ul>
<b>TSA Plus Cyanine 3.5</b>	50-150 slides	NEL763001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (15 mL)</li> <li>• Cyanine 3.5 Plus Amplification Reagent (2 tubes, add 150 µL DMSO / tube)</li> </ul>
	250-750 slides	NEL763B001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (5 x 15 mL)</li> <li>• Cyanine 3.5 Plus Amplification Reagent (10 tubes, add 150 µL DMSO / tube)</li> </ul>
	25-75 slides	NEL763E001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (7.5 mL)</li> <li>• Cyanine 3.5 Plus Amplification Reagent (1 tube, add 150 µL DMSO)</li> </ul>
<b>TSA Plus Cyanine 5</b>	50-150 slides	NEL745001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (15 mL)</li> <li>• Cyanine 5 Plus Amplification Reagent (2 tubes, add 150 µL DMSO / tube)</li> </ul>
	250-750 slides	NEL745B001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (5 x 15 mL)</li> <li>• Cyanine 5 Plus Amplification Reagent (10 tubes, add 150 µL DMSO / tube)</li> </ul>
	25-75 slides	NEL745E001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (7.5 mL)</li> <li>• Cyanine 5 Plus Amplification Reagent (1 tube, add 150 µL DMSO)</li> </ul>
<b>TSA Plus Cyanine 5.5</b>	50-150 slides	NEL766001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (15 mL)</li> <li>• Cyanine 5.5 Plus Amp Reagent (2 tubes, add 150 µL DMSO / tube)</li> </ul>
	250-750 slides	NEL766B001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (5 x 15 mL)</li> <li>• Cyanine 5.5 Plus Amplification Reagent (10 tubes, add 150 µL DMSO / tube)</li> </ul>
	25-75 slides	NEL766E001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (7.5 mL)</li> <li>• Cyanine 5.5 Plus Amplification Reagent (1 tube, add 150 µL DMSO)</li> </ul>
<b>TSA Plus Cyanine 3 / Cyanine 5</b>	50-150 slides	NEL752001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (2 x15 mL)</li> <li>• Cyanine 3 Plus Amplification Reagent (2 tubes, add 150 µL DMSO / tube)</li> <li>• Cyanine 5 Plus Amplification Reagent (2 tubes, add 150 µL DMSO / tube)</li> </ul>
<b>TSA Plus Cyanine 3 / Fluorescein</b>	50-150 slides	NEL753001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (2 x15 mL)</li> <li>• Cyanine 3 Plus Amplification Reagent (2 tubes, add 150 µL DMSO / tube)</li> <li>• Fluorescein Plus Amplification Reagent (2 tubes, add 150 µL DMSO / tube)</li> </ul>
<b>TSA Plus Cyanine 5 / Fluorescein</b>	50-150 slides	NEL754001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (2 x15 mL)</li> <li>• Cyanine 5 Plus Amplification Reagent (2 tubes, add 150 µL DMSO / tube)</li> <li>• Fluorescein Plus Amplification Reagent (2 tubes, add 150 µL DMSO / tube)</li> </ul>
<b>TSA Plus Fluorescein / Tetramethylrhodamine</b>	50-150 slides	NEL756001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (2 x15 mL)</li> <li>• TMR Plus Amplification Reagent (2 tubes, add 150 µL DMSO / tube)</li> <li>• Fluorescein Plus Amplification Reagent (2 tubes, add 150 µL DMSO / tube)</li> </ul>
<b>TSA Plus Fluorescence Palette Kit</b>	10-35 slides	NEL760001KT	<ul style="list-style-type: none"> <li>• 1X Plus Amplification Diluent (2 x15 mL)</li> <li>• Fluorescein Plus Amplification Reagent (1 tubes, add 60 µL DMSO)</li> <li>• TMR Plus Amplification Reagent (1 tube, add 60 µL DMSO)</li> <li>• Cyanine 3 Plus Amplification Reagent (1 tube, add 60 µL DMSO)</li> <li>• Cyanine 5 Plus Amplification Reagent (1 tube, add 60 µL DMSO)</li> </ul>

\*The format of the kit is based on 100 - 300 µL per slide of TSA Plus Working Solution (see page 3).

## Reagents and Materials

### Critical Reagents Required but not Supplied

- HRP-labelled reagent to drive the signal amplification reaction is required. For example:
  - Anti-digoxigenin-HRP for use with digoxigenin labeled probes or antibodies (cat. no. NEF832001EA)
  - Anti-fluorescein-HRP for use with fluorescein labeled probes or antibodies (cat. no. NEF710001EA)
  - HRP-conjugated anti-species secondary antibody
    - Anti-rabbit IgG (goat) HRP (cat. no. NEF812001EA)
    - Anti-mouse IgG (goat) HRP (cat. no. NEF822001EA)
- DMSO (molecular biology or HPLC grade)
- Buffer components and detergents such as PBS, Triton-X100, Tween-20
- Blocking Reagent (cat. no. FP1012 or equivalent)

## Solutions to prepare

The following buffers and reagents are required for slide preparation and signal amplification

### TSA Plus Stock Solution

TSA Plus Amplification Reagent is supplied as a solid. Each vial must be reconstituted with molecular biology or HPLC grade DMSO to make TSA Plus Stock Solution. TSA Plus Stock Solution is stable for at least 3 months when stored at 4°C. (Note: DMSO freezes at 4°C; therefore, thaw Stock Solution before each use). Here are recommended dissolution volumes for stock solutions.

- For the TSA Plus Fluorescence Palette Kit (NEL760001KT), add 60 µL DMSO to each tube of Amplification Reagent.
- Add 150 µL DMSO per tube Amplification Reagent for all other TSA Plus Fluorescence Kits.

### TNT Wash Buffer

0.1 M TRIS-HCl, pH 7.5

0.15 M NaCl

0.05% Tween®20

Other wash buffers (such as PBS) may be used. Substitution of 0.3% Triton X-100 for the 0.05% Tween-20 is also possible. Users should validate the use of alternative wash buffers with their own systems.

### TNB Blocking Buffer

0.1 M TRIS-HCl, pH 7.5

0.15 M NaCl

0.5% Blocking Reagent (available separately, catalog number FP1012)

Add Blocking Reagent slowly in small increments to buffer while stirring. Heat gradually to 55°C with continuous stirring to completely dissolve the Blocking Reagent. (This should take no longer than 30-60 minutes.) The solution will appear milky. Bring to room temperature before using. Aliquot and store at -20°C for long term use.

### TSA Plus Working Solution

Before each procedure, dilute TSA Plus Stock Solution 1:50 in 1X Amplification Diluent to make TSA Plus Working Solution. Approximately 100-300 µL of TSA Plus Working Solution is required per slide. Discard any unused portion of TSA Plus Working Solution.

## Recommendations

- Do not let slides dry out between steps.
- A humidified chamber is recommended for all incubation steps (i.e., a damp paper towel in a covered box).
- Drain off as much of the incubation solutions as possible, before the addition of the next solution, to prevent reagent dilution and uneven staining. Blot area around, but not on, tissue section using a tissue.
- Be sure to use enough volume of each reagent to completely cover sections or cells.
- Optional: Use of coverslips may reduce reagent evaporation, especially during steps which require long incubation at elevated temperatures (such as probe hybridization). However care must be taken upon removal to prevent damage to tissues or cells.
- If your assay includes streptavidin conjugates, check for endogenous biotin which may be a source of non-specific background.
- If there is too much signal, dilute the primary antibody, probe or HRP conjugate further. TSA Plus kits are designed for use at 1:50 dilution of TSA Plus Stock Solution for optimal results.
- First time users should apply TSA to a proven system.

## Quenching Endogenous Peroxidase

Activation and covalent binding of TSA reagent is catalyzed by peroxidase. Endogenous peroxidase will be a source of background, and should be quenched if present. Users should establish the need for doing this and optimal methodology specific to the tissues or cells being stained. Options include:

- 0.3% H<sub>2</sub>O<sub>2</sub> to 3% H<sub>2</sub>O<sub>2</sub> in PBS, incubation for 10 to 60 minutes
- 0.3% H<sub>2</sub>O<sub>2</sub> to 3% H<sub>2</sub>O<sub>2</sub> in Methanol, incubation for 10 to 60 minutes

For paraffin-embedded tissues, quenching can be done after dewaxing and alcohol rehydration but before the protease digestion step. For frozen tissue or cell preps, quenching can be done following fixation and before the protease digestion step. After quenching wash with TNT or 1X PBS buffer for 5 minutes. See Li *et al* from references section for more suggestions.

## TSA-ISH Optimization

Inadequate optimization of probe and HRP conjugate dilution may be a source of high background and reduced signal.

TSA is ideal for detection of targets that have weak signals using standard detection methods. If the signal is strong with standard detection methods, dilute the probe until the signal begins to disappear and then proceed with TSA optimization.

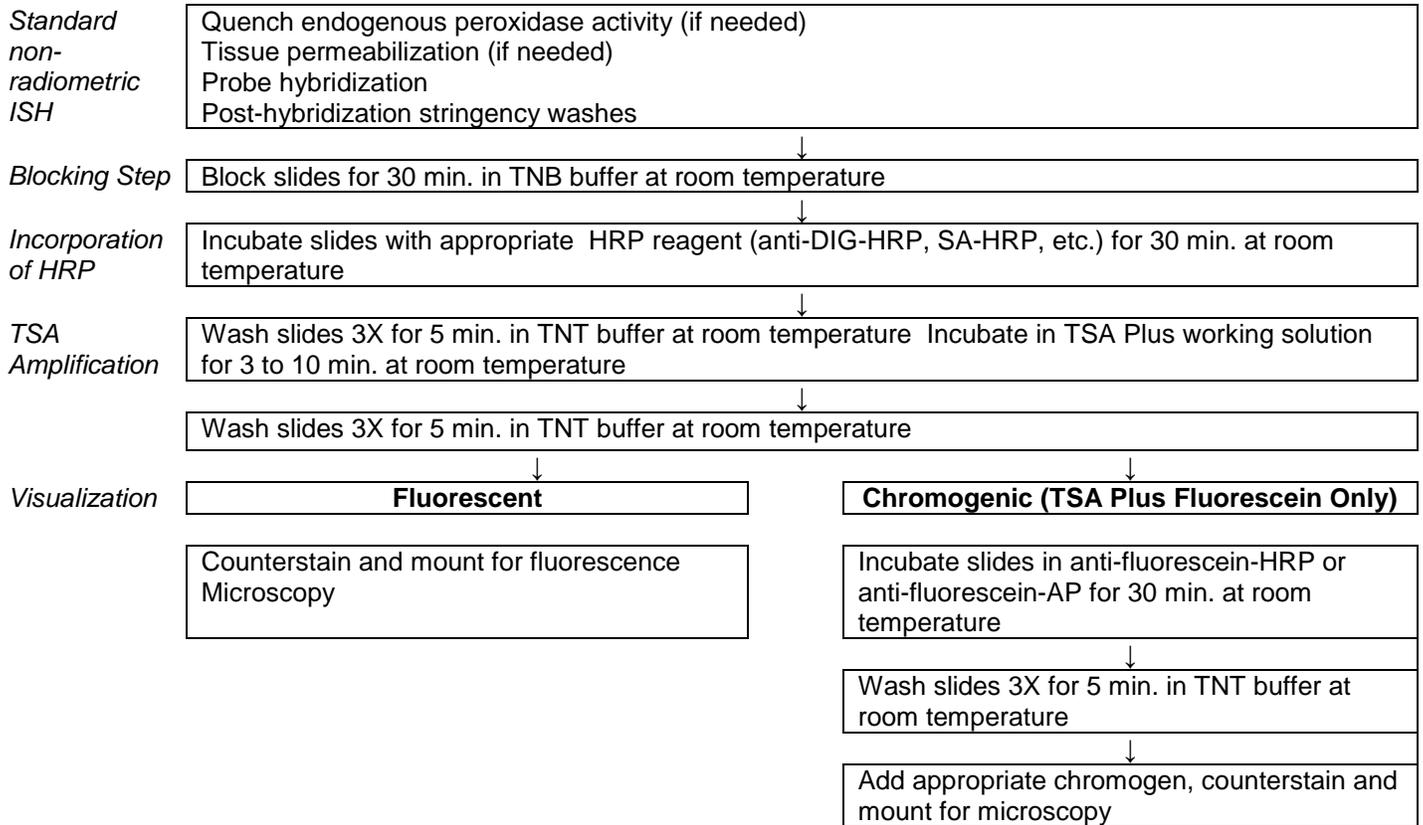
### Probe Optimization

- Slide 1: use same probe dilution as standard method.
- Slide 2: 5-fold dilution from slide 1
- Slide 3: 2-fold dilution from slide 2 (further dilution may be necessary)
- Slide 4: negative control (probe omitted)

### HRP Conjugate Dilution

- SA-HRP included with TSA Systems: 1:100 dilution
- Streptavidin-HRP (catalog number NEL750001EA): 1:250-1:1000 dilution
- Anti-fluorescein-HRP (catalog number NEF710001EA): 1:100-1:500 dilution
- Anti-DNP-HRP (catalog number FP1129): 1:100-1:500 dilution
- Anti-digoxigenin-HRP (catalog number NEF832001EA): 1:500-1:5000 dilution
- HRP reagents from other manufacturers: Begin with recommended range for slide applications. Further dilution may be needed

## TSA-ISH Protocol Overview



## Step by Step TSA-ISH Protocol

### Step 1: Slide Preparation

Prepare tissues or cells using standard fixation and embedding techniques. Dewax and rehydrate slides according to standard procedures.

### Step 2: Standard Non-radioactive ISH

Follow standard non-radioactive in situ hybridization techniques. Include tissue permeabilization (if needed) and quenching of endogenous peroxidase activity (if needed). Probe hybridization (with digoxigenin, biotin, DNP, or fluorescein-labeled probes) should be done using at concentration determined in optimization studies followed by post-hybridization stringency washes.

NOTE: Always run an unamplified control slide and an amplified negative control slide with each experiment.

### Step 3: Blocking Step

Cover tissue sections with TNB buffer (or other validated blocking buffer) and incubate slides in a humidified chamber for 30-60 minutes at room temperature or at 4°C overnight.

#### **Step 4: Introduction of HRP**

Incubate slides for 30 minutes at room temperature or at 4°C overnight in a humidified chamber with appropriate HRP-labeled reagent. Use adequate reagent volume to cover the tissue section, generally 100-300 µL per slide.

- For DIG-labeled probes, use anti-digoxigenin-HRP (catalog number NEF832001EA) diluted in TNB Buffer (or other validated blocking buffer) to concentration determined in optimization studies.
- For biotin-labeled probes, use SA-HRP (catalog number NEL750001EA) diluted in TNB Buffer (or other validated blocking buffer) to concentration determined in optimization studies.
- For fluorescein-labeled probes, use anti-fluorescein-HRP (catalog number NEF710001EA) diluted in TNB Buffer (or other validated blocking buffer) to concentration determined in optimization studies.
- For DNP-labeled probes, use anti-DNP-HRP (catalog number FP1129) diluted in TNB Buffer (or other validated blocking buffer) to concentration determined in optimization studies.

#### **Step 5: Wash**

Wash the slides 3X for 5 minutes each in TNT Buffer (or other validated wash buffer) at room temperature with agitation.

#### **Step 6: Amplification**

Pipette TSA Plus Working Solution onto each slide. Use enough working solution to completely cover the tissue section, generally 100-300 µL per slide. Incubate the slides at room temperature for 3 to 10 minutes.

#### **Step 7: Wash**

Wash the slides 3X for 5 minutes each in TNT buffer (or other validated wash buffer) at room temperature with agitation.

#### **Step 8: Visualization of Deposited Fluorophores**

For fluorescent detection, counterstain and mount for fluorescence microscopy. (See page 10 table, Fluorophore Excitation and Emission Maxima.)

For chromogenic detection with TSA Plus Fluorescein, incubate slides with anti-fluorescein-HRP (NEF710001EA) or anti-fluorescein-AP (NEF709001PK) followed by detection with an appropriate chromogenic substrate.

## **TSA-IHC Optimization**

Inadequate optimization of primary antibody and HRP conjugate dilution may be a source of high background and reduced signal.

TSA is ideal for detection of targets that have weak signals with 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.

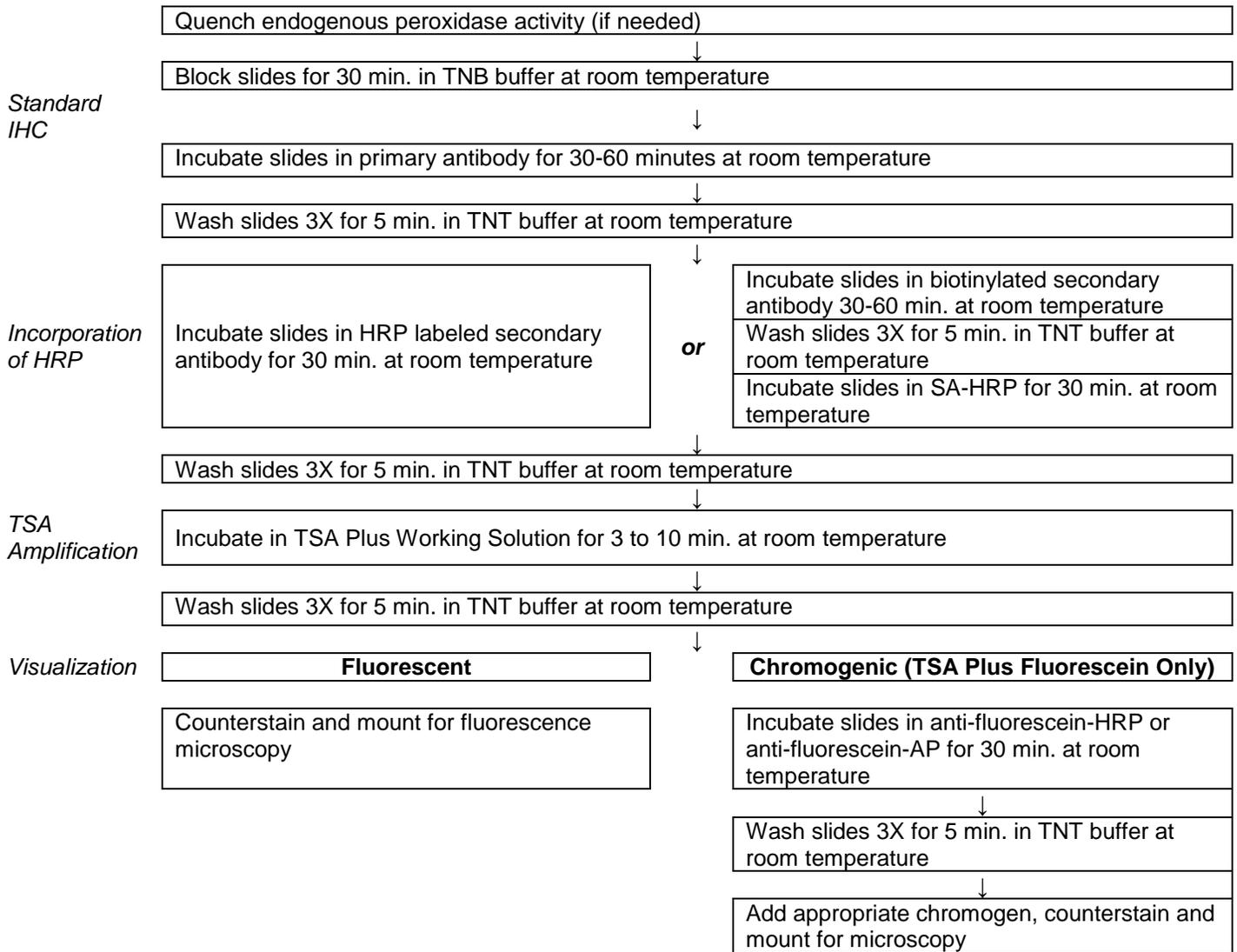
#### **Primary Antibody Optimization**

- Slide 1: use same antibody dilution as standard method.
- 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 necessary)
- Slide 4: negative control (primary antibody omitted)

#### **HRP Conjugate Dilution**

- SA-HRP included with TSA Systems: 1:100 dilution
- Anti-mouse-HRP (catalog number NEF822001EA): 1:500 to 1:2000 dilution
- Anti-rabbit-HRP (catalog number NEF812001EA): 1:500 to 1:2000 dilution
- Streptavidin-HRP (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-IHC Protocol Overview



## Step by Step TSA-IHC Protocol

### Step 1: Slide Preparation

Prepare tissues or cells for detection with TSA using standard fixation and embedding techniques. Dewax and rehydrate using standard protocols. Quench endogenous peroxidase activity if necessary.

NOTE: Always run an unamplified control slide and an amplified negative control slide with each experiment.

### Step 2: Blocking Step

Cover tissue sections with TNB buffer (or other validated blocking buffer) and incubate slides in a humidified chamber for 30-60 minutes at room temperature or at 4°C overnight.

### Step 3: Primary Antibody Incubation

Drain off the blocking buffer and apply primary antibody, diluted in TNB Buffer (or other validated blocking buffer). Incubate the primary antibody preparation per the manufacturer's instructions regarding incubation time and temperature requirements. Use enough volume to completely cover the tissue section (generally 100-300 µL per slide) at the concentration determined in optimization studies.

**Step 4: Wash**

Wash the slides 3X for 5 minutes each in TNT Buffer (or other validated wash buffer) at room temperature with agitation.

**Step 5: Introduction of HRP**

Incubate slides for 30 minutes at room temperature or at 4°C overnight in a humidified chamber with appropriate HRP-labeled reagent. Use adequate reagent volume to cover the tissue section, generally 100-300 µL per slide. Options include.

- HRP labeled secondary antibody diluted in TNB Buffer (or other validated blocking buffer).
- 100-300 µL of biotinylated secondary antibody diluted in TNB Buffer (or other validated blocking buffer). Incubate 30-60 minutes in a humidified chamber. Wash the slides for 3 X 5 minutes TNT buffer at room temperature with agitation. Follow by 100-300 µL of SA-HRP diluted in TNB Buffer. Use SA-HRP at 1:100 dilution, or at 1:2000 if using PerkinElmer Cat. # NEL750001EA.
- When using alternative suppliers, reagents should be optimized for use with TSA starting with manufacturer's recommended dilutions. Incubate slides in a humidified chamber for 30-60 minutes at room temperature or at 4°C overnight.

**Step 6: Wash**

Wash the slides 3X for 5 minutes each in TNT Buffer (or other validated wash buffer) at room temperature with agitation.

**Step 7: Amplification**

Pipette 100-300 µL of TSA Plus Working Solution onto each slide. Incubate the slides at room temperature for 3 to 10 minutes.

**Step 8: Wash**

Wash the slides 3X for 5 minutes each in TNT Buffer (or other validated wash buffer) at room temperature with agitation.

**Step 9: Visualization of Deposited Fluorophores**

For fluorescent detection, counterstain and mount for fluorescence microscopy. (See page 10 table, Fluorophore Excitation and Emission Maxima.)

For chromogenic detection with TSA Plus Fluorescein, incubate slides with anti-fluorescein-HRP (NEF710001EA) or anti-fluorescein-AP (NEF709001PK) followed by detection with an appropriate chromogenic substrate.

## Troubleshooting

### Technical Support Resources

- Assay Support Knowledge Base: [www.perkinelmer.com/askTSA](http://www.perkinelmer.com/askTSA)
- Email: [global.techsupport@perkinelmer.com](mailto:global.techsupport@perkinelmer.com)
- Telephone
  - USA toll-free **800-762-4000**
  - EU toll-free **00800 33 29 0000**
  - Finland toll-free **999 800 33 29 0000**
  - China toll-free **800 820 5046**

### ISH Troubleshooting

PROBLEM	REMEDY
Low Signal	<ul style="list-style-type: none"> <li>• Optimize probe concentration.</li> <li>• Titer HRP conjugate to determine optimum concentration for signal amplification.</li> <li>• Add tissue permeabilization step to facilitate penetration of reagents.</li> <li>• Lengthen incubation time for TSA Plus Working Solution.</li> </ul>
Excess Signal	<ul style="list-style-type: none"> <li>• Decrease concentration of HRP conjugate introduced prior to amplification.</li> <li>• Decrease probe concentration.</li> <li>• Decrease TSA Plus Working Solution incubation time.</li> <li>• Decrease concentration of anti-fluorescein-enzyme conjugate used for chromogenic visualization.</li> </ul>
High Background	<ul style="list-style-type: none"> <li>• Decrease probe concentration.</li> <li>• Decrease concentration of HRP conjugate.</li> <li>• Check for endogenous biotin (if using streptavidin conjugates)</li> <li>• Shorten chromogenic development time.</li> <li>• Lengthen endogenous peroxidase quenching step.</li> <li>• Filter buffers.</li> <li>• Increase number and/or length of washes.</li> <li>• Nonqualified or contaminated blocking reagent used. Use PerkinElmer Blocking Reagent (FP1020 or FP1012).</li> </ul>

### IHC Troubleshooting

PROBLEM	REMEDY
Low Signal	<ul style="list-style-type: none"> <li>• Titer primary and/or secondary antibodies to determine optimum concentration for signal amplification</li> <li>• Lengthen incubation time for TSA Plus Working Solution.</li> <li>• Use antigen retrieval techniques to unmask the target.</li> </ul>
Excess Signal	<ul style="list-style-type: none"> <li>• Decrease concentration of primary and/or secondary antibody or HRP conjugates.</li> <li>• Decrease TSA Plus Working Solution incubation time.</li> <li>• Decrease concentration of anti-fluorescein-enzyme conjugate used for chromogenic visualization.</li> </ul>
High Background	<ul style="list-style-type: none"> <li>• Filter buffers</li> <li>• Decrease concentration of primary and/or secondary antibody or HRP conjugates.</li> <li>• Lengthen endogenous peroxidase quenching step.</li> <li>• Check for endogenous biotin (if using streptavidin conjugates)</li> <li>• Increase number and/or length of washes.</li> <li>• Shorten chromogenic development time.</li> <li>• Nonqualified or contaminated blocking reagent used. Use PerkinElmer Blocking Reagent (FP1020 or FP1012).</li> </ul>

## Selected References

Brend, Tim, and Scott A. Holley. "Zebrafish whole mount high-resolution double fluorescent in situ hybridization." *Journal of visualized experiments: JoVE* 25 (2009).

Brown, Jason, Hallie Wimberly, Donald R. Lannin, Christian Nixon, David L. Rimm, and Veerle Bossuyt. "Multiplexed Quantitative Analysis of CD3, CD8, and CD20 Predicts Response to Neoadjuvant Chemotherapy in Breast Cancer." *Clinical Cancer Research* (2014): clincanres-1622.

Lee, Seung-won, Song Eun Lee, Seong Hyuk Ko, Eun Kyoung Hong, Kwang Il Nam, Kei-ichiro Nakamura, Shuhei Imayama et al. "Introduction of tyramide signal amplification (TSA) to pre-embedding nanogold-silver staining at the electron microscopic level." *Journal of Histochemistry & Cytochemistry* 53, no. 2 (2005): 249-252.

Lein, Ed S., Michael J. Hawrylycz, Nancy Ao, Mikael Ayres, Amy Bensinger, Amy Bernard, Andrew F. Boe et al. "Genome-wide atlas of gene expression in the adult mouse brain." *Nature* 445, no. 7124 (2006): 168-176.

Liu, Gang, Sejal Amin, Nataly N. Okuhama, Guoning Liao, and Lisa A. Mingle. "A quantitative evaluation of peroxidase inhibitors for tyramide signal amplification mediated cytochemistry and histochemistry." *Histochemistry and cell biology* 126, no. 2 (2006): 283-291.

Silahtaroglu, Asli N., Dorrit Nolting, Lars Dyrskjøt, Eugene Berezikov, Morten Møller, Niels Tommerup, and Sakari Kauppinen. "Detection of microRNAs in frozen tissue sections by fluorescence in situ hybridization using locked nucleic acid probes and tyramide signal amplification." *Nature protocols* 2, no. 10 (2007): 2520-2528.

Stack, Edward C., Chichung Wang, Kristin Roman, and Clifford C. Hoyt. "Multiplexed immunohistochemistry, imaging, and quantitation: a review, with an assessment of Tyramide signal amplification, multispectral imaging and multiplex analysis." *Methods* (2014).

Zaidi, Aliya U., Hideki Enomoto, Jeffrey Milbrandt, and Kevin A. Roth. "Dual fluorescent in situ hybridization and immunohistochemical detection with tyramide signal amplification." *Journal of Histochemistry & Cytochemistry* 48, no. 10 (2000): 1369-1375.

## TSA Fluorophore Excitation and Emission Maxima

Fluorophore	Excitation	Emission
Coumarin	402 nm	443 nm
Fluorescein	494 nm	517 nm
Tetramethylrhodamine	550 nm	570 nm
Cyanine 3	550 nm	570 nm
Cyanine 3.5	581 nm	596 nm
Cyanine 5	648 nm	667 nm
Cyanine 5.5	673 nm	692 nm

## Complementary Products

TSA Blocking Reagent	3 g	FP1020
	10 g	FP1012
<b>Horseradish Peroxidase Reagents</b>		
Anti-rabbit IgG (goat) HRP	1 mL	NEF812001EA
Anti-rabbit IgG (goat) HRP	100 µL	NEF812E001EA
Anti-mouse IgG (goat) HRP	1 mL	NEF822001EA
Anti-mouse IgG (goat) HRP	100 µL	NEF822E001EA
Anti-human IgG (goat)* HRP	1 mL	NEF802001EA
Anti-DNP-HRP	150 µL	FP1129
Antifluorescein-HRP	2x250µL	NEF710001EA
Streptavidin-HRP	2x250µL	NEL750001EA
Anti-digoxigenin HRP	500 µL	NEF832001EA
<b>Biotin Conjugates</b>		
Anti-rabbit IgG (goat) biotin	1 mL	NEF813001EA
Anti-mouse IgG (goat) biotin	1 mL	NEF823001EA
Anti-human IgG (goat) biotin	1 mL	NEF803001EA
Anti-digoxigenin biotin	500 µL	NEF833001EA
<b>Labeled Streptavidin</b>		
Streptavidin Fluorescein	1 mL	NEL720001EA
Streptavidin Texas Red®	1 mL	NEL721001EA
Streptavidin Coumarin	1 mL	NEL722001EA
Streptavidin-HRP	2x250µL	NEL750001EA
Streptavidin-AP	2x250µL	NEL751001EA
<b>Chromogens</b>		
BCIP/NBT Substrate	For detection of Alkaline Phosphatase	NEL937001PK
DAB Substrate	For detection of Horseradish Peroxidase	NEL938001EA
<b>Alkaline Phosphatase Reagents</b>		
Anti-Mouse IgG (Goat), AP-Labeled	1 mL	NEF814001EA
Anti-Rabbit IgG (Goat), AP-Labeled	1 mL	NEF824001EA
Streptavidin- AP Conjugate	2x250µL	NEL751001EA
Antifluorescein-AP Conjugate	2x250µL	NEF709001PK
Anti-DNP-AP	150 µL	FP1131

<b>Hapten Labeled Deoxynucleotides (25 nmol, for labeling of ISH probes)</b>		
3-Amino-3-Deoxydigoxigenin-9-dCTP	25 nmol	NEL562001EA
Biotin-11-dATP	25 nmol	NEL540001EA
Biotin-11-dCTP	25 nmol	NEL538001EA
Biotin-11-dGTP	25 nmol	NEL541001EA
Biotin-11-dUTP	25 nmol	NEL539001EA
DNP-11-dUTP	25 nmol	NEL551001EA
Fluorescein-12-dATP	25 nmol	NEL465001EA
Fluorescein-12-dCTP	25 nmol	NEL424001EA
Fluorescein-12-dGTP	25 nmol	NEL429001EA
Fluorescein-12-dUTP	25 nmol	NEL413001EA
<b>Hapten Labeled Ribonucleotides (25 nmol, for labeling of ISH probes)</b>		
Biotin-11-ATP	250 nmol	NEL544001EA
Biotin-11-CTP	250 nmol	NEL542001EA
Biotin-11-GTP	250 nmol	NEL545001EA
Biotin-11-UTP	250 nmol	NEL543001EA
Fluorescein-12-ATP	250 nmol	NEL439001EA
Fluorescein-12-CTP	250 nmol	NEL434001EA
Fluorescein-12-GTP	250 nmol	NEL496001EA
Fluorescein-12-UTP	250 nmol	NEL414001EA

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