MANUAL

TSA® Research Reagents

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

TSA Biotin Kits

Material Provided

	Format*	Catalog #	Kit Components
TSA Biotin Systems (includes blocking reagent and SA-HRP)	50-150 slides	NEL700A001KT	• 1X Amplification Diluent (15 mL) • Biotin Tyramide (dry, dissolve in 300 µL DMSO) • Streptavidin-HRP (150 µL) • Blocking Reagent (3 g)
	200-600 slides	NEL700001KT	• 1X Amplification Diluent (30 mL) • Biotin Tyramide (dry, dissolve in 1200 µL DMSO) • Streptavidin-HRP (2 x 150 µL) • Blocking Reagent (10 g)
TSA Biotin Stand Alone Tyramide Kits	200-600 slides	SAT700001KT	• 1X Amplification Diluent (60 mL) • Biotin Tyramide (dry, dissolve in 1200 µL DMSO)
	1000-3000 slides	SAT700B001KT	• 1X Amplification Diluent (4 x 75 mL) • Biotin Tyramide (5 vials, dry, dissolve in 1200 µL DMSO / vial)

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

Product Information

Storage: Store kits in the dark at 4ºC.

Stability: The components in the kits are stable for a minimum of 6 months under proper storage conditions.

Application: TSA Biotin 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.

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?

Tyramide Signal Amplification (TSA™) from PerkinElmer is a technology that improves sensitivity by up to 100-fold while allowing reduced consumption of primary antibodies or probes.

TSA Biotin Kits use horseradish peroxidase (HRP) to catalyze covalent deposition of biotin labels directly adjacent to the immobilized enzyme. The labeling reaction is quick (less than 10 minutes) and deposited labels can be detected with streptavidin conjugates for imaging in brightfield or fluorescence 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.

Reagents and Materials

Critical Reagents Required but not Supplied

- Depending upon your assay, additional HRP-labelled reagent to drive the signal amplification reaction may be required. For example:
    - Streptavidin-HRP for use with biotin labeled probes or antibodies (cat. no. NEL750001EA)
    - 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)
- Streptavidin enzyme or fluorophore conjugate for visualization of biotin labels.
- 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

Biotin Tyramide Stock Solution

Biotin Tyramide Reagent is supplied as a solid. Each vial must be reconstituted with DMSO or water as indicated to make Biotin Tyramide Stock Solution. Biotin Tyramide Stock Solution is stable for at least 3 months when stored at 4ºC. (Note: DMSO freezes at 4ºC; therefore, thaw Stock Solution before each use). Here are recommended dissolution volumes for stock solutions.

Catalog #	Tyramide Stock Solution Preparation
NEL700A001KT	Add 300 µL DMSO to reagent tube
NEL700001KT	Add 1200 µL DMSO to reagent tube
SAT700001KT	Add 1200 µL DMSO to reagent tube
SAT702001KT	Add 1200 µL DMSO to each reagent tube

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.

Biotin Tyramide Working Solution
Before each procedure, dilute Biotin Tyramide Stock Solution 1:50 in 1X Amplification Diluent to make Biotin Tyramide Working Solution. Approximately 100-300 µL of Biotin Tyramide Working Solution is required per slide. Discard any unused portion of Biotin Tyramide 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).
- Before the addition of the next solution, drain off as much of the previous solution as possible to prevent reagent dilution and uneven staining. After draining the solution, use a Kimwipe™ or similar lint free tissue to blot the area around (but not on) the tissue section.
- 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.
- 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 kits are designed for use at 1:50 dilution of the tyramide reagent 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_2O_2 to 3% H_2O_2 in PBS, incubation for 10 to 60 minutes
- 0.3% H_2O_2 to 3% H_2O_2 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

Standard non-radiometric ISH: Quench endogenous peroxidase activity (if needed); Tissue permeabilization (if needed); Probe hybridization; Post-hybridization stringency washes

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Blocking Step: Block slides for 30 min. in TNB buffer at room temperature

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Incorporation of HRP: Incubate slides with appropriate HRP reagent (anti-DIG-HRP, SA-HRP, etc.) for 30 min. at room temperature

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TSA Amplification: Wash slides 3X for 5 min. in TNT buffer at room temperature Incubate in tyramide working solution for 3 to 10 min. at room temperature

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

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Visualization

Fluorescent	**Chromogenic (TSA Fluorescein Only)**
Incubate slides in SA-fluorophore for 30 min. at room temperature.	Incubate slides in streptavidin-HRP or streptavidin-AP for 30 min. 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	Add appropriate chromogen, counterstain and mount for microscopy

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 4ºC overnight.

Step 4: Introduction of HRP
Incubate slides for 30 minutes at room temperature or at 4ºC 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 NEF710001EA) 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 Biotin Tyramide 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 Biotin

Deposited biotin may be visualized for fluorescent or chromogenic detection.

For fluorescent detection, incubate slides with fluorophore labeled streptavidin for 30 minutes at room temperature or as directed by the manufacturer. Counterstain and mount for fluorescence microscopy.

For chromogenic detection, incubate slides with HRP or AP labeled streptavidin 30 minutes at room temperature or as directed by the manufacturer. Visualize with an appropriate chromogen as directed by the manufacturer. Counterstain and mount for microscopy.

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.

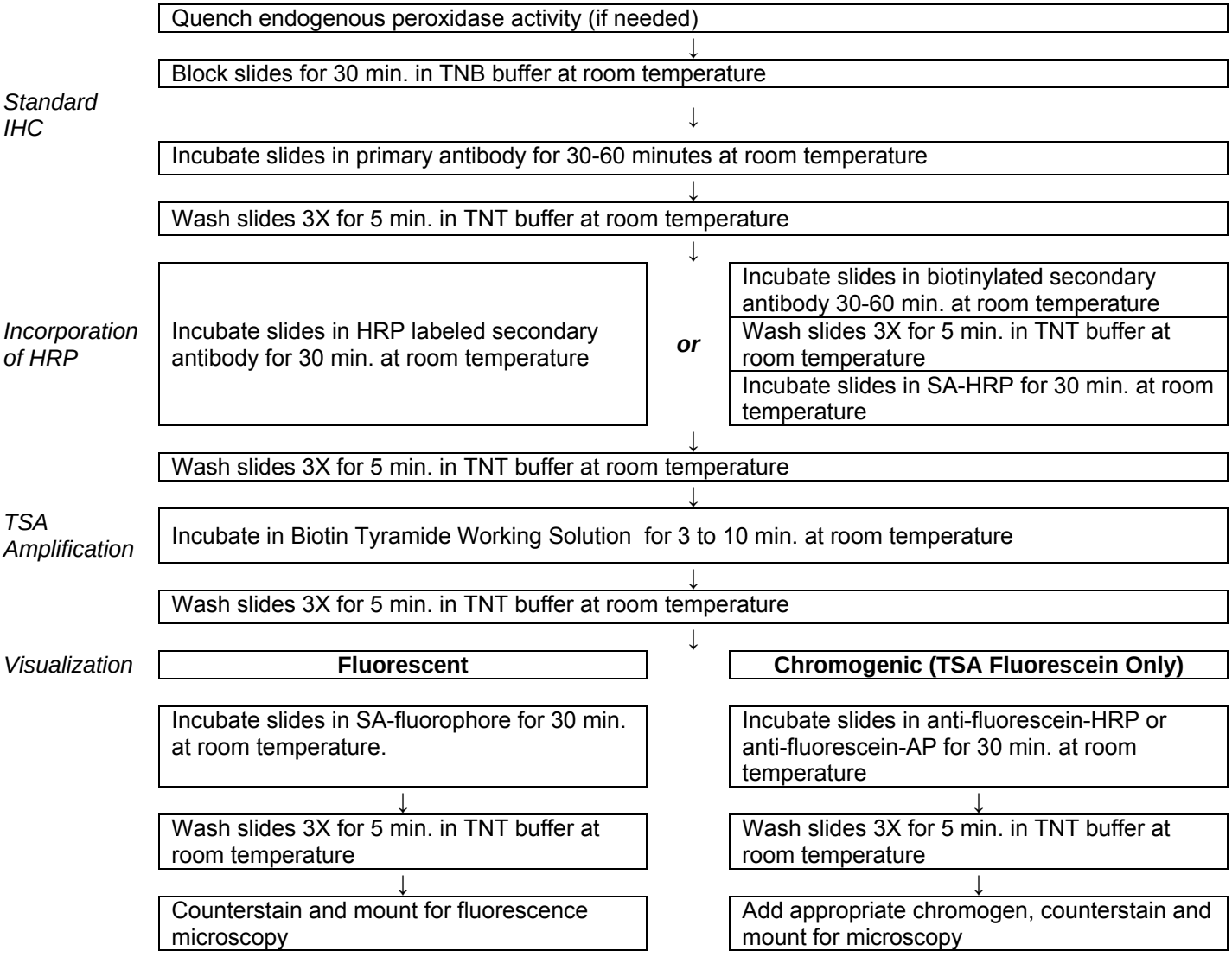
Primary Antibody Optimization

- 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 4ºC 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 4ºC 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 4ºC 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 Biotin Tyramide 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

Deposited biotin may be visualized for fluorescent or chromogenic detection.

For fluorescent detection, incubate slides with fluorophore labeled streptavidin for 30 minutes at room temperature or as directed by the manufacturer. Counterstain and mount for fluorescence microscopy.

For chromogenic detection, incubate slides with HRP or AP labeled streptavidin 30 minutes at room temperature or as directed by the manufacturer. Visualize with an appropriate chromogen as directed by the manufacturer. Counterstain and mount for microscopy.

Troubleshooting

Technical Support Resources

- **Assay Support Knowledge Base:** **www.perkinelmer.com/askTSA**
- **Email:** **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	• Optimize probe concentration. • Titer HRP conjugate to determine optimum concentration for signal amplification. • Add tissue permeabilization step to facilitate penetration of reagents. • Lengthen incubation time for Biotin Tyramide Working Solution. • Consider TSA Plus reagents for higher signal.
Excess Signal	• Decrease concentration of HRP conjugate introduced prior to amplification. • Decrease probe concentration. • Decrease Biotin Tyramide Working Solution incubation time. • Decrease concentration of streptavidin-enzyme conjugate used for chromogenic visualization.
High Background	• Decrease probe concentration. • Decrease concentration of HRP conjugate. • Check for endogenous biotin • Shorten chromogenic development time. • Lengthen endogenous peroxidase quenching step. • Filter buffers. • Increase number and/or length of washes. • Nonqualified or contaminated blocking reagent used. Use PerkinElmer Blocking Reagent (FP1020 or FP1012).

IHC Troubleshooting

PROBLEM	REMEDY
Low Signal	• Titer primary and/or secondary antibodies to determine optimum concentration for signal amplification • Lengthen incubation time for Biotin Tyramide Working Solution. • Use antigen retrieval techniques to unmask the target. • Consider TSA Plus reagents for higher signal.
Excess Signal	• Decrease concentration of primary and/or secondary antibody or HRP conjugates. • Decrease Biotin Tyramide Working Solution incubation time. • Decrease concentration of streptavidin-enzyme conjugate used for chromogenic visualization..
High Background	• Filter buffers • Decrease concentration of primary and/or secondary antibody or HRP conjugates. • Lengthen endogenous peroxidase quenching step. • Check for endogenous biotin • Increase number and/or length of washes. Shorten chromogenic development time. • Nonqualified or contaminated blocking reagent used. Use PerkinElmer Blocking Reagent (FP1020 or FP1012).

Selected References

A quantitative evaluation of peroxidase inhibitors for tyramide signal amplification mediated cytochemistry and histochemistry
G. Li, S. Amin, N. N. Okuhama, G. Liao, L. A. Mingle
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Zebrafish Whole Mount High-Resolution Double Fluorescent In Situ Hybridization
Tim Brend, Scott A. Holley
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Neuron. (2008)
http://www.ncbi.nlm.nih.gov/sites/entrez/19109908?dopt=Abstract&holding=f1000,f1000m,isrctn

Detection of microRNAs in frozen tissue sections by fluorescence in situ hybridization using locked nucleic acid probes and tyramide signal amplification
Asli N Silahtaroglu, Dorrit Nolting, Lars Dyrskjøt, Eugene Berezikov, Morten Møller, Niels Tommerup & Sakari Kauppinen
Nat Protoc. (2007)
http://www.ncbi.nlm.nih.gov/pubmed/17947994

Introduction of Tyramide Signal Amplification (TSA) to Pre-embedding Nanogold–Silver Staining at the Electron Microscopic Level
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J Histochem Cytochem. (2005)
http://www.ncbi.nlm.nih.gov/pubmed/15684338

Complementary Products

TSA Blocking Reagent	3 g	FP1020
	10 g	FP1012
Horseradish Peroxidase Reagents		
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-digoxigigenin HRP	500 µL	NEF832001EA
Biotin Conjugates		
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-digoxigigenin biotin	500 µL	NEF833001EA
Labeled Streptavidin		
Streptavidin Fluorescein	1 mL	NEL720001EA
Streptavidin Texas Red®	1 mL	NEL721001EA
Streptavidin Coumarin	1 mL	NEL722001EA
Streptavidin-HRP	2x250µL	NEL750001EA
Streptavidin-AP	2x250µL	NEL751001EA
Chromogens		
BCIP/NBT Substrate	For detection of Alkaline Phosphatase	NEL937001PK
DAB Substrate	For detection of Horseradish Peroxidase	NEL938001EA
Alkaline Phosphatase Reagents		
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

Hapten Labeled Deoxynucleotides (25 nmol, for labeling of ISH probes)		
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
Hapten Labeled Ribonucleotides (25 nmol, for labeling of ISH probes)		
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