TSA™ PLUS Biotin KIT

Tyramide **S**ignal **A**mplification

For in Situ *Hybridization and Immunohistochemistry*

NEL749A001KT TSA Plus Biotin

Kit (50 - 150 slides*)

NEL749B001KT TSA Plus Biotin

Kit (250 - 750 slides*)

* number of slides determined by volume used per section

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I. INTRODUCTION

A. Background Information

What is ISH?

In situ hybridization (ISH) is a technique used to detect, visualize and localize DNA and RNA at the cellular level. Labeled probes are hybridized to nucleic acid targets in tissue or cell preps. Probes can be labeled using common labeling techniques such as in vitro transcription, nick translation, and 3' end labeling. Detection schemes for in situ hybridization include autoradiography for radioactive probes, dye deposition for enzyme or hapten-labeled probes, and fluorescence for fluorophore-labeled probes.

What is IHC?

Immunohistochemistry (IHC) is a technique to detect, visualize and localize antigens at the cellular level. Common IHC protocols use primary and secondary antibodies to indirectly detect antigens in frozen or paraffin-embedded tissue sections. Detection schemes for IHC include dye deposition for enzyme labeled antibodies, fluorescence for fluorescent labeled antibodies and silver enhancement for systems using gold labeling.

What is TSA?

TSA™ (Tyramide Signal Amplification) is a powerful, patented technology from PerkinElmer that significantly enhances both chromogenic and fluorescent signals. It is easily integrated into standard nonradioactive ISH or IHC protocols, provided that horseradish peroxidase (HRP) is in the system.

How does TSA™ Plus Biotin Signal Amplification work?

TSA Plus Biotin technology uses HRP to catalyze the covalent deposition of biotin labeled amplification reagent onto tissue sections or cell preparation surfaces that have been previously blocked with proteins. The reaction is quick (less than 10 minutes) and results in the covalent deposition of numerous biotin labels immediately adjacent to the immobilized HRP enzyme. These labels can then be indirectly detected by standard chromogenic or fluorescent techniques, with significant enhancement of the signal. Detection is accomplished through the use of a streptavidin with an enzyme or fluorophore conjugate. Because the added labels are deposited proximal to the initial immobilized HRP enzyme site, there is minimal loss in resolution. This signal amplification technique may be applied to both ISH and IHC.

What ISH and IHC mediums are compatible with TSA Plus Biotin?

TSA Plus Biotin has been successfully applied to the following media: formalin-fixed/paraffin-embedded sections, cryostat sections, and cultured cells.

B. The TSA Plus Biotin Kit

The TSA Plus Biotin Kit contains the following components necessary for signal amplification:

- Biotin Amplification Reagent,
- 1X Plus Amplification Diluent

Intended Use

The intended use of this kit is to amplify signals generated by Horseradish Peroxidase in nonradioactive ISH and IHC applications. The reagents in this kit have been optimized for use in slide based assays. These kits are not suitable for use on membranes or microtiter plates. Labeled streptavidin, blocking reagent and other components should be purchased separately.

FOR LABORATORY USE.

Safety Note

All reagents are classified as nonhazardous. We strongly recommend wearing disposable gloves and safety glasses while working. Thorough washing of hands after handling is also recommended. Do not eat, smoke, or drink in areas in which reagents are handled.

C. Components of TSA Plus Biotin Kits

NEL749A001KT 50-150 slides *

Reagent	Amount
1X Plus Amplification Diluent	15 mL
Biotin Amplification Reagent	1 vial

NEL749B001KT 250-750 slides *

Reagent	Amount
1X Plus Amplification Diluent	5x15 mL
Biotin Amplification Reagent	5 vial

Biotin Amplification Reagent is supplied as a solid.

* The number of slides is determined by the reagent volume (approximately 100-300 uL) which is needed to completely cover the cells or tissue section on the slide.

Storage and Stability

Upon receipt, the TSA Plus Biotin kit should be stored at 4°C. The components in this kit are stable for 6 months under proper storage conditions. Do not use beyond expiration date

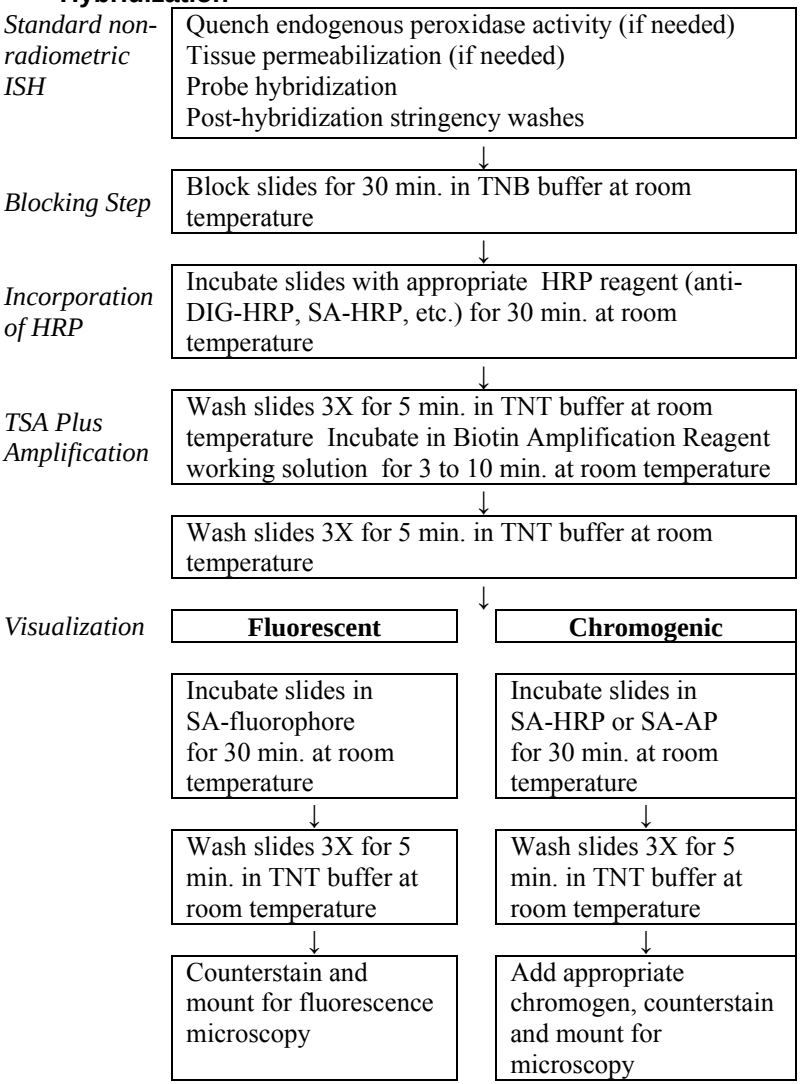
listed on kit.

Critical Reagents Required But Not Supplied

- HRP-labeled reagent, for example
    - SA-HRP for use with biotin-labeled probes or antibodies (PerkinElmer cat. no. NEL750001EA)
    - HRP-labeled secondary antibody
    - Anti-digoxigenin-HRP for use with DIG-labeled probes (for example Roche Anti-Digoxigenin-POD cat. no. 11 207 733 910)
- DMSO (molecular biology or HPLC grade)
- Buffer components
- Blocking reagent (PerkinElmer cat. no. FP1020)
- Labeled streptavidin or anti-biotin
- Chromogenic substrate as appropriate (BCIP/NBT, DAB, AEC, etc.)

II. PROTOCOL FOR ISH

A. Overview Protocol for TSA Plus Biotin for In Situ Hybridization



B. Suggested ISH Protocol

The following is a suggested protocol for using TSA Plus Biotin Kit for in situ hybridization signal amplification.

TSA technology requires HRP to be an integral part of the detection protocol. This may be accomplished via the use of various hapten-labeled probe/anti-hapten-HRP conjugate combinations such as digoxigenin-labeled probe followed by anti-DIG-HRP, biotin-labeled probe followed by SA-HRP, or with a fluorescein-labeled probe followed by anti-fluorescein-HRP. Once HRP is introduced, the Biotin Amplification Reagent working solution is added. Visualization is done through the use of appropriate enzyme/chromogen combinations. . Chromogenic substrates must be bought separately.

First Time Users

First time nonradioactive ISH users should assess the need for various tissue pre-treatment conditions which may be necessary to improve penetration of reagents and/or to reduce background. A balance must be achieved between making the target accessible versus causing loss of target and/or destruction of tissue morphology. Reagent penetration may be improved by protein digestion or detergent permeabilization prior to probe hybridization. Common protein digestion methods include the use of 0.005-0.1% pepsin in 0.01M HCl or Proteinase K (1-10 µ g/mL) in TRIS-HCl / 0.05M EDTA. Cell preparations are often permeabilized with detergents such as saponin or Triton X-100. Background may be reduced

using procedures such as acetylation of tissue and/or inhibition of endogenous enzyme (peroxidase or alkaline phosphatase) activity.

First time **TSA Plus Biotin** users **should apply this to a proven ISH system.**

Controls

Always run control slides with each experiment! These should include an unamplified control slide (i.e., include specific probe but eliminate TSA regents) and an amplified negative control (i.e., no probe or non-specific probe + TSA reagents) slide. In addition to proving validity of results, control slides are often beneficial in determining the cause of non-specific background.

Reagent Titration

In general, researchers have found that TSA requires lower probe and conjugate concentrations for optimal results when compared with standard unamplified nonradioactive methods:

1. Probe titration:

Probe concentration must be optimized. It should be assessed using the standard concentration used in unamplified nonradiometric procedures, and at reduced concentrations of 5, 10, and 20-fold - less in the hybridization mix. In general, a 10-fold reduction

in probe concentration has most often been found to be optimal. **Failure to establish appropriate probe concentration can result in little to no signal development.**

2. Titration of enzyme conjugates:

Incubation with an appropriate HRP conjugate is required for the activation of the Biotin amplification reagent. Appropriate HRP conjugate concentrations to assess include supplier's recommended starting concentration, 2-fold less, and 5-fold less. In cases where no signal and no background are seen, it may be necessary to use an increased concentration instead. For example, if the recommended starting titer is 1:100, run slides with HRP conjugate at 1:50, 1:100, 1:200, and 1:500.

Quenching Endogenous Peroxidase

Activation and covalent binding of the Biotin Amplification Reagent is catalyzed by HRP. To minimize background, endogenous peroxidase activity, if present, must be quenched. 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
- Methanol or PBS as diluent for H_2O_2
- Incubation time of 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 TN or 1X PBS buffer for 5 minutes. See Li *et al* from references section for more suggestions.

Failure to establish optimum tissue pre-treatments and reagent concentrations may result in poor signal amplification and/or increased background.

Volumes

The protocol is written for minimal volumes of reagent (e.g., 100-300 µL). Reagent volumes used should be sufficient to completely cover cells or tissue sections on slide. If larger volumes are necessary to cover the samples, make the appropriate changes in the protocol.

Technical Support

If there are any further questions regarding TSA in your ISH system, please contact PerkinElmer Technical Support Department before proceeding. Refer to the Troubleshooting Guide section for local listing. Technical assistance is also available through our web site at www.perkinelmer.com/tsa.

C. Standard ISH Protocol

1. Preparation of Buffers and Reagents

The following buffers and reagents are required for slide preparation and TSA Plus Biotin amplification.

Biotin Amplification Reagent Stock Solution

Biotin Amplification Reagent is supplied as a solid and may be difficult to see in the vial. Reconstitute by adding 0.3 mL/vial of DMSO (dimethyl sulfoxide -molecular biology or HPLC-grade) to make the Biotin Amplification Reagent Stock Solution. Biotin Amplification Reagent Stock Solution, when stored at 4° C, is stable for at least six 6 months. (Note: DMSO should freeze at 4° C. Thaw the Stock Solution, before each use.)

Biotin Amplification Reagent Working Solution

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

Wash Buffer

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.

Blocking Buffer

TNB Blocking Buffer

0.1 M TRIS-HCl, pH 7.5

0.15 M NaCl

0.5% Blocking Reagent (available separately, catalog number FP1020)

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 temperatue befor using. Aliquot and store at -20°C for long term use.

2. Procedural Notes

- 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.

3. Step by Step Protocol

The following is a ***suggested*** protocol for the use of TSA Plus Biotin in an 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 Technique

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, or fluorescein-labeled probes) should be done using aconcentration determined in optimization studies (see p. 9) 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

Incubate slides with 100-300 µL of TNB Buffer in a

humidified chamber for 30-60 minutes at room temperature or at 4^0C overnight.

Step 4: Introduction of HRP

Incubate slides for 30 minutes at room temperature or at 4^0C overnight. in a humidified chamber with appropriate HRP-labeled reagent using either:

a. DIG-labeled probes: 100-300 µL of anti-digoxigenin-HRP (Roche anti-DIG-POD Cat. No. 11 207 733 910) diluted 1:100 in TNB Buffer,

or

b. Biotin-labeled probes: 100-300 µL of SA-HRP (PerkinElmer Cat. No. NEL750001EA) diluted 1:2000 in TNB Buffer.

or

c. Fluorescein-labeled probes: 100-300 µL of anti-fluorescein-HRP (PerkinElmer Cat. No. NEF710001EA) diluted 1:250 in TNB Buffer.

NOTE: HRP-labeled reagents are available from a variety of vendors. Appropriate concentration for use should be established as per optimization studies suggested on p.9).

Step 5: Wash

Wash the slides 3X for 5 minutes each in

TNT Buffer at room temperature with agitation.

Step 6: TSA Plus Biotin Amplification

Pipet 100-300 µL of the Biotin Amplification Reagent Working Solution (p. 11) onto each 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 at room temperature with agitation.

Step 8: Visualization of Deposited Biotin

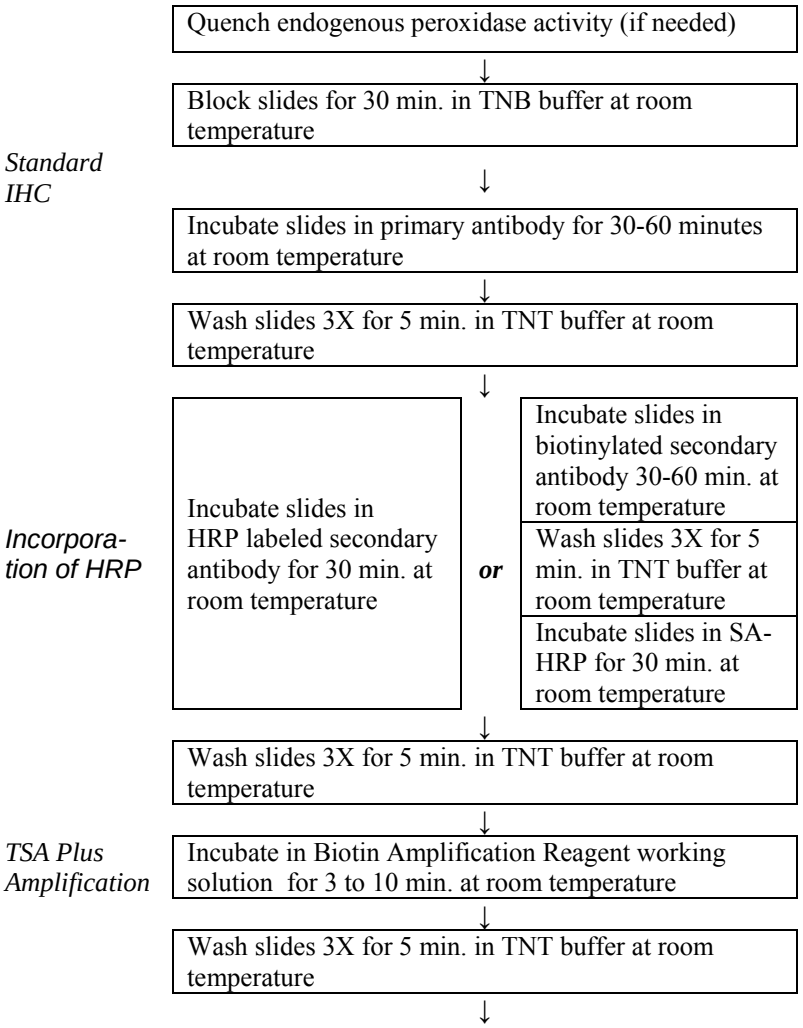
Deposited biotin may be visualized for fluorescent or chromogenic detection.

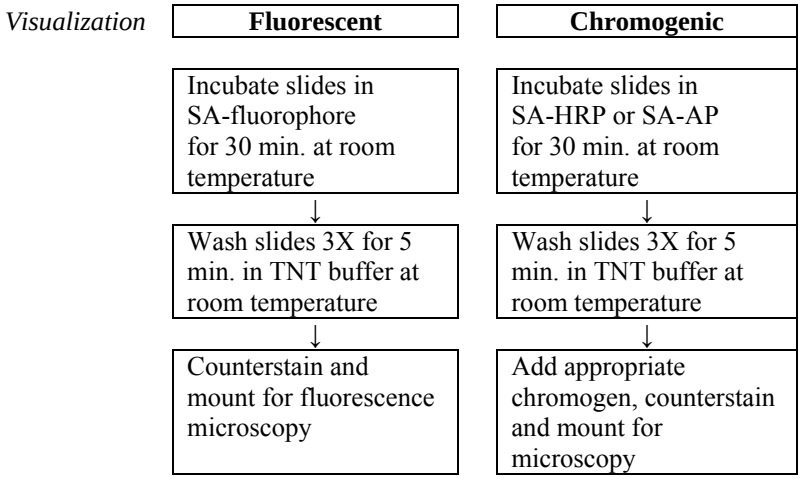
For fluorescent detection, incubate slides with fluorophore labeled streptavidin as directed by the manufacturer.

For chromogenic detection, incubate slides with HRP or AP labeled streptavidin followed by detection with an appropriate chromogenic substrate.

III. PROTOCOL FOR IHC

A. Overview Protocol for TSA Plus Biotin Immunohistochemistry





B. Suggested IHC Protocol

The following is a ***suggested*** protocol for using TSA Plus Biotin for immunohistochemistry signal amplification.

TSA technology requires HRP to be an integral part of the detection protocol. This may be accomplished by using either an HRP labeled secondary antibody or a hapten labeled secondary antibody followed by an appropriate HRP conjugate. Once HRP is introduced, the Biotin Amplification Reagent Working Solution is added. Visualization is done through the use of appropriate labeled streptavidin for fluorescent or chromogenic detection.

First Time Users

First time users should apply TSA Biotin Plus to a proven IHC

system.

Controls

Always run control slides with each experiment. Include at least one negative control slide (eliminating primary antibody but including the **TSA Plus** reagents) and one unamplified control slide (include all reagents except **TSA-Plus**). In addition to proving validity of results, control slides may be beneficial in determining the cause of non-specific background.

Reagent Titration

Failure to establish optimum reagent concentrations may result in poor amplification and/or increased background. Primary and/or secondary antibody dilutions should be optimized when applying TSA for the first time. The following test slides are recommended:

Test slide 1:

Primary or Secondary Ab at manufacturer's recommended dilution.

Test slide 2:

5 fold dilution of slide #1 Ab concentration.

Test slide 3:

5 fold dilution of slide #2 Ab concentration.

Test slide 4:

5 fold dilution of slide #3 Ab concentration.

Test slide 5:

Unamplified control.

More than the above dilutions may be necessary. In cases where low signal is obtained, **increasing the dilution** of the primary antibody often leads to better signal amplification. In many cases, the optimal dilution of the primary antibody for TSA detection is 1000-fold less than that used for standard detection.

Quenching Endogenous Peroxidase

Activation and covalent binding of the **Biotin Amplification Reagent** is catalyzed by HRP. To minimize background, endogenous peroxidase activity, if present, must be quenched before the immunostaining protocol. 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
- Methanol or PBS as diluent for H_2O_2
- Incubation time of 10 to 60 minutes

For paraffin-embedded tissues quenching can be done after dewaxing and alcohol rehydration but before the blocking step. For frozen tissue or cell preps, quenching can be done following fixation and before the blocking step. See Li *et al* from the references for more suggestions.

After quenching wash with TNT buffer for 5 minutes.

Volumes

The protocol is written for minimal volumes of reagent (e.g., 100-300 µL). Reagent volumes used should be sufficient to completely cover cells or tissue sections on slide. If larger volumes are necessary to cover the samples, make the appropriate changes in the protocol.

Technical Support

If there are any further questions regarding TSA in your IHC system, please contact PerkinElmer Technical Support Department before proceeding. Refer to the Troubleshooting Guide section for local listing. Technical assistance is also available through our web site at www.perkinelmer.com/tsa.

C. Standard IHC Protocol

1. Preparation of Buffers and Reagents

The following buffers and reagents are required for slide preparation and TSA Plus Biotin amplification.

Biotin Amplification Reagent Stock Solution

Biotin Amplification Reagent is supplied as a solid. Reconstitute by adding 0.3 mL/vial of dimethyl sulfoxide (DMSO-molecular biology or HPLC-grade) to make the Biotin Amplification Reagent Stock Solution. Biotin Amplification Reagent Stock Solution, when stored at 4° C, is stable for at least six 6 months. (Note: DMSO should freeze at 4° C. Thaw the Stock Solution before each use.)

Biotin Amplification Reagent Working Solution

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

Wash Buffer

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 wash buffer alternatives with their own systems.

NOTE: When staining cell surface/membrane targets, do NOT include detergent in wash buffer or diluents. Detergents may cause stripping or alteration of cell surface antigens.

Blocking Buffer

TNB Blocking Buffer

0.1 M TRIS-HCl, pH 7.5

0.15 M NaCl

0.5% Blocking Reagent (available separately, catalog number FP1020)

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 look milky. . Bring to room temperature before using.

Aliquot and store at -20°C for long term use.

2. Procedural Notes

- 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 dilution and uneven staining. Blot area around, but not on, tissue section using a tissue.
- Be sure to use enough volume of solutions to cover sections or cells.
- Optional: Use of coverslips may reduce reagent evaporation. However care must be taken upon removal to prevent damage to tissues or cells.

3. Step by Step Protocol

The following is a ***suggested*** protocol for the use of **TSA Plus Biotin** in IHC applications.

Step 1: Slide Preparation

Prepare tissues or cells for detection with **TSA Plus Biotin** 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

Incubate slides with 100-300 µL of TNB Buffer in a humidified chamber for 30-60 minutes at room temperature or at 4^0C overnight. (Note: PBS may be substituted for the TN buffer.)

Step 3: Primary Antibody Incubation

Drain off the TNB Buffer and apply 100-300 µL of the primary antibody, diluted in TNB Buffer. Incubate the primary antibody preparation per the manufacturer's instructions regarding incubation time and temperature requirements. Use concentration determined in optimization studies (see p.19).

Step 4: Wash

Wash the slides 3X for 5 minutes each in

TNT Buffer at room temperature with agitation.

Step 5: Introduction of HRP

Incubate slides with HRP by doing one of the following:

a) 100-300 µL of HRP labeled secondary antibody diluted in TNB Buffer.

or

b) 100-300 µL of biotinylated secondary antibody diluted in TNB 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 1:2000 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 4^0C overnight.

Step 6: Wash

Wash the slides 3X for 5 minutes each in TNT Buffer at room temperature with agitation.

Step 7: TSA Plus Biotin Amplification

Pipette 100-300 µL of the Biotin Amplification Working Solution (p. 21) 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 inTNT Buffer at room

temperature with agitation.

Step 9: Visualization of Deposited Biotin

Deposited biotin may be visualized with fluorescent or chromogenic detection.

For fluorescent detection, incubate slides with fluorophore labeled streptavidin as directed by the manufacturer.

For chromogenic detection, incubate slides with HRP or AP labeled streptavidin followed by detection with an appropriate chromogenic substrate.

IV. TROUBLESHOOTING GUIDE

A. In Situ Hybridization (ISH)

PROBLEM	REMEDY
Low Signal	• Titer HRP conjugate used for visualization to determine optimum concentration for signal amplification. • Increase concentration of Biotin Amplification Reagent solution and/or lengthen incubation time. • Add tissue permeabilization step to facilitate penetration of reagents.
Excess Signal	• Decrease concentration of HRP conjugate introduced prior to amplification. • Decrease probe concentration. • Decrease Biotin Amplification Reagent incubation time. Decrease concentration of streptavidin-enzyme conjugate used for visualization.
High Background	• Decrease concentration of HRP conjugate. • Decrease probe concentration. • Shorten chromogenic development time. • Lengthen endogenous peroxide quenching step. • Samples may contain endogenous biotin. Switch to fluorescein or digoxigenin labeled probes. • Filter buffers. • Increase number and/or length of washes. • Nonqualified or contaminated blocking reagent used. Use PerkinElmer Blocking Reagent (FP1020).

B. Immunohistochemistry (IHC)

PROBLEM	REMEDY
Low Signal	• Titer primary and/or secondary antibodies to determine optimum concentration for signal amplification • Increase concentration of Biotin Amplification Reagent solution and/or increase incubation time. • In some cases it may be necessary to utilize antigen retrieval techniques to unmask the target.
Excess Signal	• Decrease concentration of primary and/or secondary antibody or HRP conjugates. • Decrease Biotin Amplification Reagent incubation time. • Decrease concentration of Biotin conjugates used for visualization.
High Background	• Filter buffers • Decrease concentration of primary and/or secondary antibody or HRP conjugates. • Lengthen endogenous peroxide quenching step. • Increase number and/or length of washes. Shorten chromogenic development time. • Nonqualified or contaminated blocking reagent used. Use PerkinElmer Blocking Reagent (FP1020).

C. Customer Technical Support Services

For Further Technical Information, or, to Place an Order Contact:

Web site: http://www.perkinelmer.com/

Email: TechSupport@perkinelmer.com

In the U.S.:	PerkinElmer Technical Support Department at (800) 762-4000.
Outside the U.S.:	Contact your local PerkinElmer sales office or distributor.

V. Ordering information

TSA Plus Kits: 10 to 20 times more sensitive than regular TSA (includes labeled amplification reagent and amplification diluent)		
Product	No. of Slides	Product No.
TSA Plus Fluorescence Kits		
TSA Plus Fluorescein Kit	50–150	NEL741001KT
	250–750	NEL741B001KT
TSA Plus TMR Kit	50–150	NEL742001KT
	250–750	NEL742B001KT
TSA Plus Cyanine 3 Kit	50–150	NEL744001KT
	250–750	NEL744B001KT
TSA Plus Cyanine 5 Kit	50–150	NEL745001KT
	250–750	NEL745B001KT
TSA Plus Multi-Fluor Combination Kits		
TSA Plus Cyanine 3/Cyanine 5 Kit	50–150	NEL752001KT
TSA Plus Cyanine 3/Fluorescein Kit	50–150	NEL753001KT
TSA Plus Cyanine 5/Fluorescein Kit	50–150	NEL754001KT
TSA Plus Fluorescein/TMR Kit	50–150	NEL756001KT
TSA Plus Fluorescence Palette Kit (contains one each of Fluorescein, TMR, Cyanine 3 and Cyanine 5)	10–35	NEL760001KT
TSA Plus Biotin Kits		
TSA Plus Biotin Kit	50–150	NEL749A001KT
	250–750	NEL749B001KT

TSA Plus DNP Systems: sensitive, biotin-free chromogenic detection (includes DNP amplifcation reagent , amplification diluent, anti-DNP enzyme conjugate and blocking reagent)		
Product	No. of Slides	Product No.
TSA Plus DNP (AP) System	25–75	NEL746B001KT
	50–150	NEL746A001KT
TSA Plus DNP (HRP) System	25–75	NEL747B001KT
	50–150	NEL747A001KT
TSA Fluorescence Systems (includes labeled amplification reagent, amplification diluent, streptavidin HRP and blocking reagent)		
Product	No. of Slides	Product No.
TSA Fluorescein System	50–150	NEL701A001KT
	100–300	NEL701001KT
TSA TMR System	100–300	NEL702001001KT
TSA Coumarin System	100–300	NEL703001KT
TSA Cyanine 3 System	50–150	NEL704A001KT
TSA Cyanine 5 System	50–150	NEL705A001KT
TSA Biotin Systems (includes labeled amplification reagent, amplification diluent, streptavidin HRP and blocking reagent)		
TSA Biotin System	50–150	NEL700A001KT
	200–600	NEL700001KT

TSA Reagent Packs (includes labeled tyramide and amplification diluent)		
Product	No. of Slides	Product No.
TSA Biotin Tyramide Reagent Pack	200–600	SAT700001EA
	1,000–3,000	SAT700B001EA
TSA Fluorescein Tyramide Reagent Pack	100–300	SAT701001EA
	500–1,500	SAT701B001EA
TSA TMR Tyramide Reagent Pack	100–300	SAT702001EA
TSA Cyanine 3 Tyramide Reagent Pack	50–150	SAT704A001EA
	250–750	SAT704B001EA
TSA Cyanine 5 Tyramide Reagent Pack	50–150	SAT705A001EA

Complementary Products		
TSA Blocking Reagent		FP1020
Horseradish Peroxidase Reagents		
Anti-rabbit IgG (goat) HRP		NEF812001EA
Anti-mouse IgG (goat) HRP		NEF822001EA
Anti-human IgG (goat)* HRP		NEF802001EA
Anti-DNP-HRP		FP1129
Antifluorescein-HRP		NEF710001EA
Streptavidin-HRP		NEL750001EA
Biotin Conjugates		
Anti-rabbit IgG (goat) biotin		NEF813001EA
Anti-mouse IgG (goat) biotin		NEF823001EA
Anti-human IgG (goat) biotin		NEF803001EA

Labeled Streptavidin		
Streptavidin Fluorescein		NEL720001EA
Streptavidin Texas Red®		NEL721001EA
Streptavidin Coumarin		NEL722001EA
Streptavidin-HRP		NEL750001EA
Streptavidin-AP		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		NEF814001EA
Anti-Rabbit IgG (Goat), AP-Labeled		NEF824001EA
Streptavidin- AP Conjugate		NEL751001EA
Antifluorescein-AP Conjugate		NEF709001PK
Anti-DNP-AP		FP1131

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

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