



Tyramide Signal Amplification (TSA) Technical Manual

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Technical Background

Traditional pathology serves as the foundation of disease diagnosis, relying on qualitative and semi-quantitative morphological evaluation of tissue sections to identify pathological changes. Although single-plex immunohistochemistry (IHC) enables specific detection of individual biomarkers, it is limited to single-target analysis and cannot fully characterize the complexity of the tissue microenvironment.

To address this critical limitation of conventional brightfield staining, multiplex fluorescence staining has been developed and widely adopted. Instead of traditional chromogenic dyes, antibodies are conjugated to distinct fluorophores with unique emission spectra. Under fluorescence microscopy, multiple target signals can be clearly distinguished and spatially localized, making this approach well-suited for dissecting the spatial distribution and cellular interaction of multiple molecular biomarkers.

Nevertheless, conventional multiplex fluorescence staining still exhibits notable technical limitations. It frequently suffers from insufficient signal intensity, impairing the detection of low-abundance targets. Spectral overlap and channel crosstalk frequently interfere with pathological interpretation. In addition, fluorophores are susceptible to photobleaching, leading to compromised signal stability and limiting long-term imaging as well as sample data preservation.

The advent of tyramide signal amplification (TSA) addresses these challenges, providing a robust enzymatic signal amplification strategy for multiplex fluorescence staining. By fundamentally overcoming the bottlenecks of conventional detection methods, TSA enables high-sensitivity, high-specificity, and high-stability multiplex fluorescence in situ detection.

Technical Introduction

Tyramide Signal Amplification (TSA) is a highly sensitive in situ detection technology based on enzymatic catalytic reaction. Its core mechanism relies on the high catalytic activity of horseradish peroxidase (HRP) to achieve specific and high-density labeling of target proteins and nucleic acid molecules in biological samples. As a pivotal approach to break the sensitivity bottleneck of conventional detection methods, TSA substantially enhances the fluorescence intensity of target molecules across major applications, including immunocytochemistry (ICC), immunohistochemistry (IHC), and fluorescence in situ hybridization (FISH).

Compared with traditional analytical methods, TSA exhibits prominent technical advantages.

First, it provides exceptional signal amplification, enabling reliable detection of low-abundance targets undetectable by conventional approaches-such as weakly expressed functional proteins, rare cell-surface markers, and low-copy nucleic acid fragments.

Second, it supports robust multiplex labeling. The tyramide polymerization reaction maintains strict regional specificity to avoid signal crosstalk, and is compatible with a variety of fluorescent dyes, allowing simultaneous multi-target labeling and panoramic interpretation of molecular profiles.

Third, the protocol is mild and tissue-friendly. An optimized mild elution buffer enables multiple cycles of sequential staining without high-temperature processing, preserving tissue integrity and antigenicity, improving experimental reproducibility, and maximizing the utility of precious samples.

Fourth, it exhibits broad compatibility with multiple sample formats, including paraffin sections, frozen tissues, cell preparations and organoids. It is fully compatible with conventional imaging systems and circumvents the host species restriction of secondary antibodies, substantially reducing technical barriers to application.

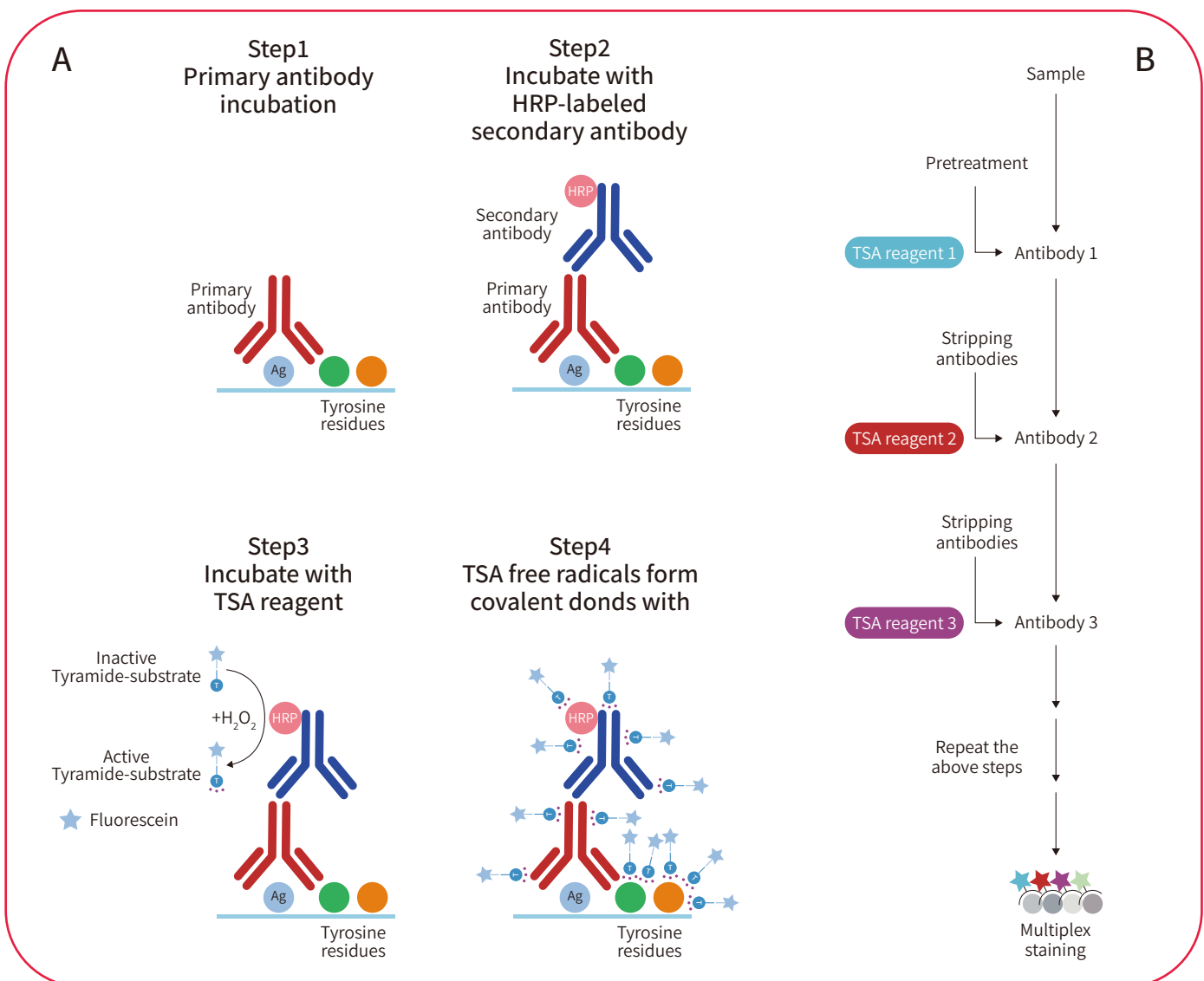
Collectively, TSA provides an accurate and comprehensive in situ detection solution for life science research and clinical diagnosis, and offers strong technical support for basic research as well as precision clinical diagnostics.

Detection Principle

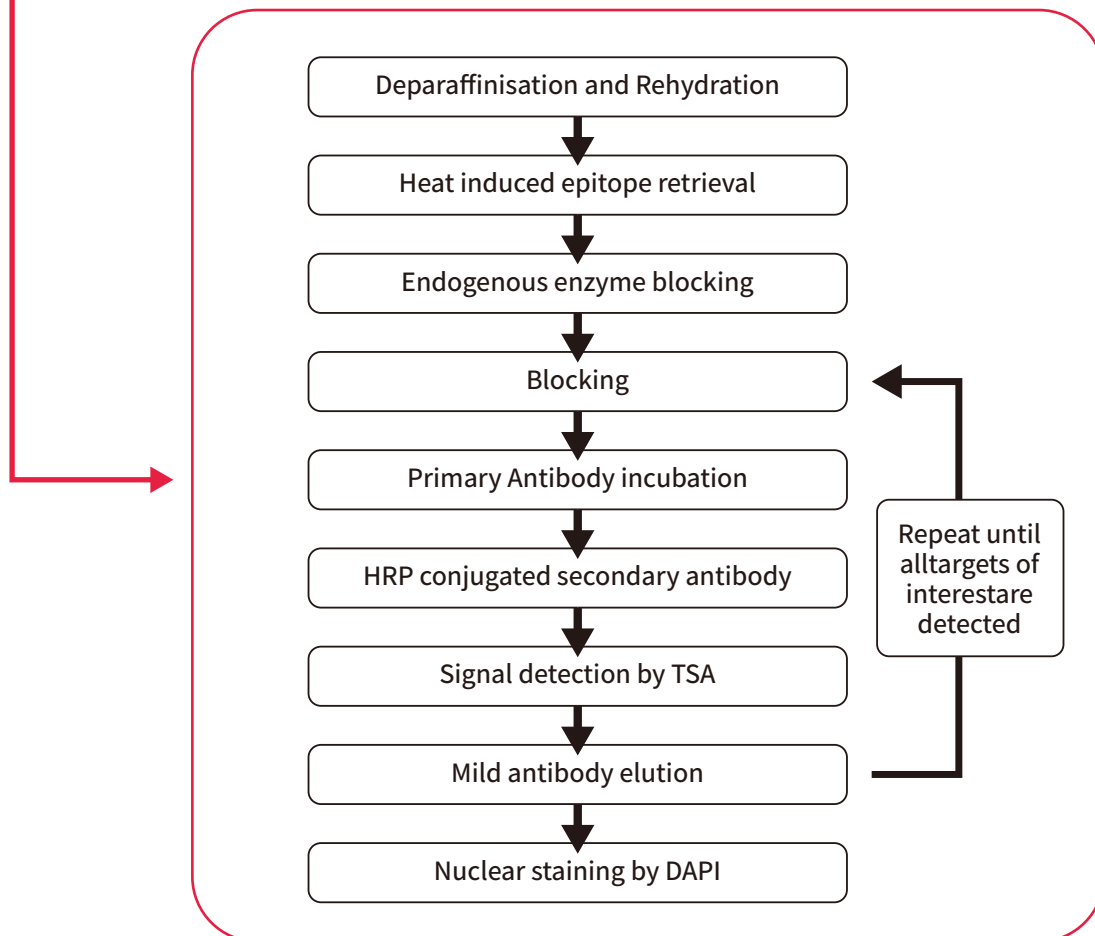
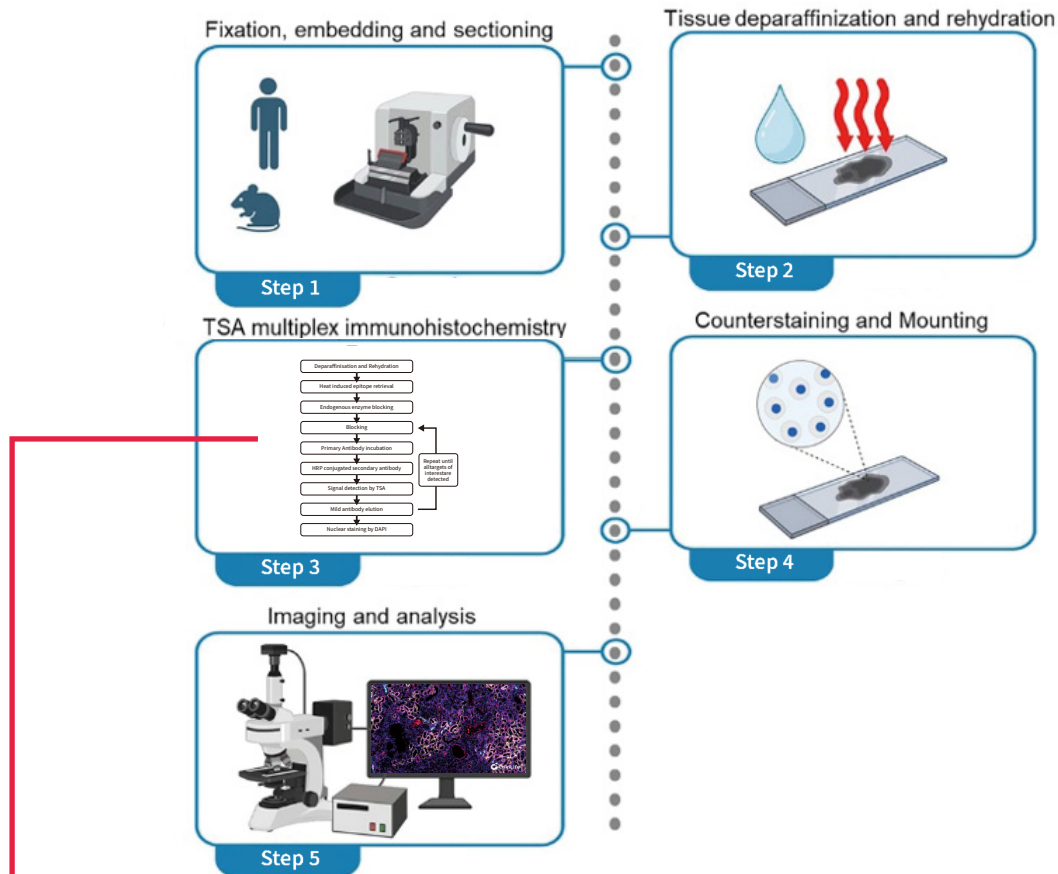
TSA technology utilizes tyramide salts to form covalent binding sites under the catalysis of horseradish peroxidase (HRP) and hydrogen peroxide, producing abundant enzymatic products. These products covalently attach to adjacent amino acid residues (tryptophan, histidine, and tyrosine) in proteins, resulting in massive fluorophore deposition at antigen–antibody binding sites and amplifying detection signals 10–100-fold.

In brief, HRP conjugated on the secondary antibody catalyzes inactive fluorophore-tyramide substrates added into the reaction system. Activated tyramide fluorophores covalently couple with tyrosine residues of adjacent proteins, enabling stable immobilization of fluorescence signals within the tissue sample. Afterwards, non-covalently bound primary antibody–secondary antibody–HRP complexes are thoroughly eluted. Another round of incubation is performed with a new pair of primary and HRP-conjugated secondary antibodies, followed by a different fluorophore-tyramide substrate. Repeating this cyclic procedure enables high-plex multiple biomarker labeling.

Since only one primary antibody is used per staining cycle, antibody cross-reactivity is effectively minimized, and strict species-matching constraints between primary and secondary antibodies are eliminated. This greatly simplifies antibody selection and experimental design. With TSA technology, all targets on the same tissue section can be detected using highly specific rabbit monoclonal primary antibodies, paired with a single universal anti-rabbit HRP secondary antibody, while achieving substantial signal amplification.



Operation Process



Key technical points

A. Antigen Retrieval

Antigen retrieval is routinely performed using 1× citrate buffer (pH 6.0) under high-temperature and high-pressure. Place the slides in a pressure cooker with an appropriate amount of retrieval buffer, seal the cooker, start timing for 2 minutes once steam pressure is reached, then cool to room temperature to complete retrieval. For weakly expressed biomarkers: Use EDTA buffer (pH 9.0). For fragile tissues prone to section detachment such as bone and brain tissues, adopt microwave antigen retrieval at approximately 80 °C, with 2× citrate buffer (pH 6.0) as the retrieval solution.

B. Endogenous Enzyme Blocking

Prepare 3% hydrogen peroxide (H₂O₂) solution with purified water, immerse the slides in the solution and incubate at room temperature for 20 minutes. For tissues prone to detachment, appropriately reduce the H₂O₂ concentration and incubation duration. For specimens containing abundant hemoglobin (e.g., liver and spleen tissues), extend the incubation time as appropriate to eliminate autofluorescence induced by hemoglobin.

C. Antibody Selection and Dilution

Monoclonal antibodies are preferred over polyclonal antibodies. For mouse tissue samples, avoid using mouse-derived primary antibodies. If mouse primary antibodies are adopted, the secondary antibody may bind not only to the primary antibody but also to endogenous tissue IgG, resulting in non-specific staining. The optimal dilution ratio of the primary antibody should be determined by preliminary trials. High concentrations cause non-specific binding; low concentrations yield weak signals. It is recommended to set up a concentration gradient to screen the optimal working condition.

D. Specifications for TSA Dye Application

Fluorescent dyes are light-sensitive; prepare working solutions fresh. For low-abundance targets, select dyes with high fluorescence intensity and strong anti-photobleaching performance (e.g., Cy5, AF594) to improve detection sensitivity via TSA signal amplification. For high-abundance targets, choose dyes with moderate fluorescence intensity (e.g., FITC, Cy3) to prevent signal saturation and loss of histological details.

E. Avoidance of Spectral Overlap

Select dye combinations with an emission wavelength interval of ≥ 50 nm. Typical combination: DAPI (461 nm) + AF488 (519 nm) + Cy3 (570 nm) + Cy5 (670 nm), which can effectively reduce spectral crosstalk. Do not use FITC (520 nm) together with AF488 (519 nm), or Cy3 (570 nm) together with AF594 (617 nm).

F. Compatibility with Imaging Equipment

For fluorescent TSA staining, verify that the fluorescence channels match the wavelength configuration of the laboratory microscope. For conventional fluorescence microscopes, prefer visible-spectrum dyes such as FITC, Cy3 and DAPI, and avoid far-red dyes such as Cy5. For confocal or multispectral microscopes, far-red dyes (Cy5, AF647) are applicable; multispectral unmixing technology can further reduce fluorescence crosstalk.

G. Antibody Elution Efficiency

High-titer and high-affinity antibodies may be difficult to elute completely; increase the elution cycles if necessary. The antibody elution buffer has good fluidity. If slides are not placed horizontally, the solution will flow out of the circled tissue area and compromise elution efficiency. Always keep slides flat during operation. For specimens prone to section detachment, lower the temperature of elution buffer or shorten the incubation time.

Typical Application Scenarios

The advantages of TSA technology are not merely theoretical; its outstanding performance in practical applications further confirms its necessity for widespread adoption:

A.Tumor Immune Microenvironment Research

High-impact studies use TSA multiplex fluorescence to simultaneously label tumor cells, immune checkpoint molecules, and infiltrating immune cell markers. TSA overcomes the limitations of conventional IHC, including single-target detection, weak signal intensity, and the inability to achieve co-localization. It has been widely applied in exploring immune escape mechanisms, immunotherapy resistance mechanisms, and screening prognostic biomarkers.

B.Clinical Pathological Diagnosis and Subtyping

TSA detects low-abundance biomarkers in formalin-fixed paraffin-embedded (FFPE) sections. It is applied to precise tumor classification, identification of early minimal lesions, and pathological interpretation of difficult cases, effectively compensating for the insufficient sensitivity of routine IHC.

C.Neuroscience and Neurodegenerative Disease Research

Multiple neuroscience studies utilize TSA to detect low-abundance functional proteins, neuronal markers, and glial cell phenotyping in brain tissues. Compatible with both frozen and paraffin-embedded brain sections, TSA supports research on Alzheimer's disease, brain injury, and neuronal regulatory mechanisms.

D.Stem Cells, Organoids and Developmental Biology

Studies on organoids and stem cell differentiation commonly apply TSA for multi-target lineage labeling, enabling cell subtype localization and differentiation trajectory tracing. Benefiting from minimal sample consumption and compatibility with multiple rounds of cyclic staining, TSA greatly preserves valuable organoid samples.

E.Low-Copy Fluorescence in Situ Hybridization (FISH)

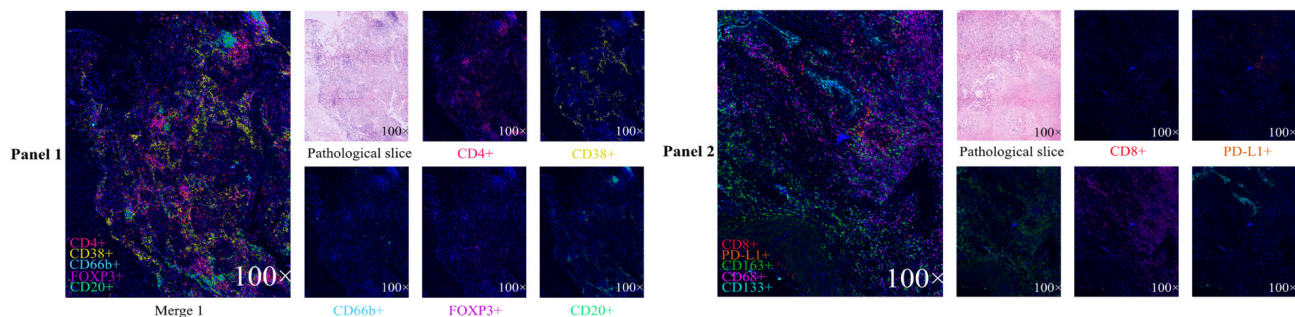
As documented in FISH methodological literature, TSA can amplify the weak signals of nucleic acid hybridization. It is suitable for the detection of gene copy number variation, in situ localization of viral nucleic acids, and analysis of trace RNA expression.



Examples of TSA Multiplex Immunofluorescence Results

1

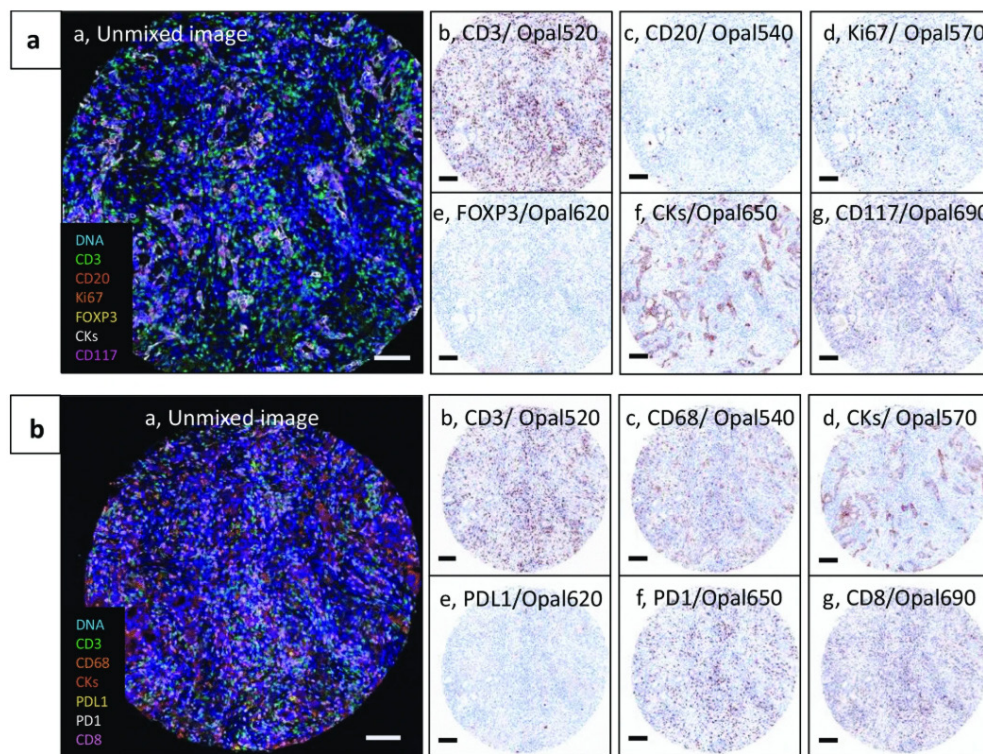
Example 1: Multicolor fluorescence labeling for delineating tumor immune landscape and cellular interaction network of NSCLC



Expression profile of 10 immune biomarkers in non-small cell lung cancer^[1].

2

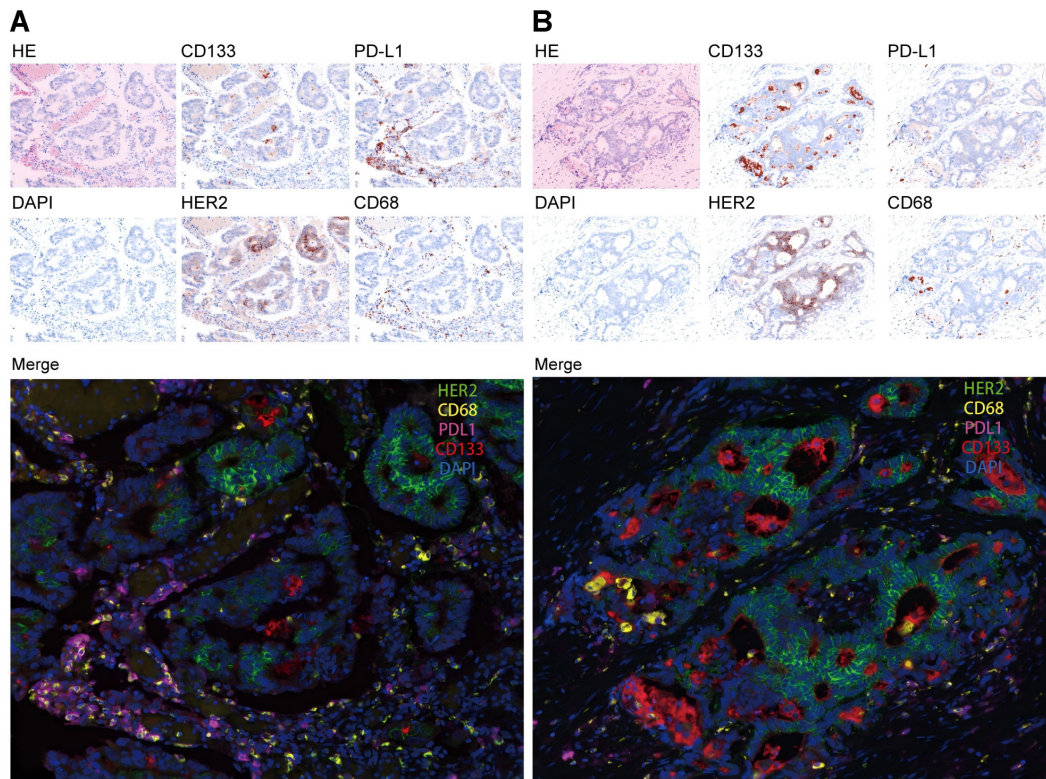
Example 2: Multiplex immunofluorescence combined with tissue microarray for systematic analysis of TME cellular composition, functional status and immune molecular interactions in various tumors



A human bronchial airway biopsy TMA was stained with mIHC^[2].

3

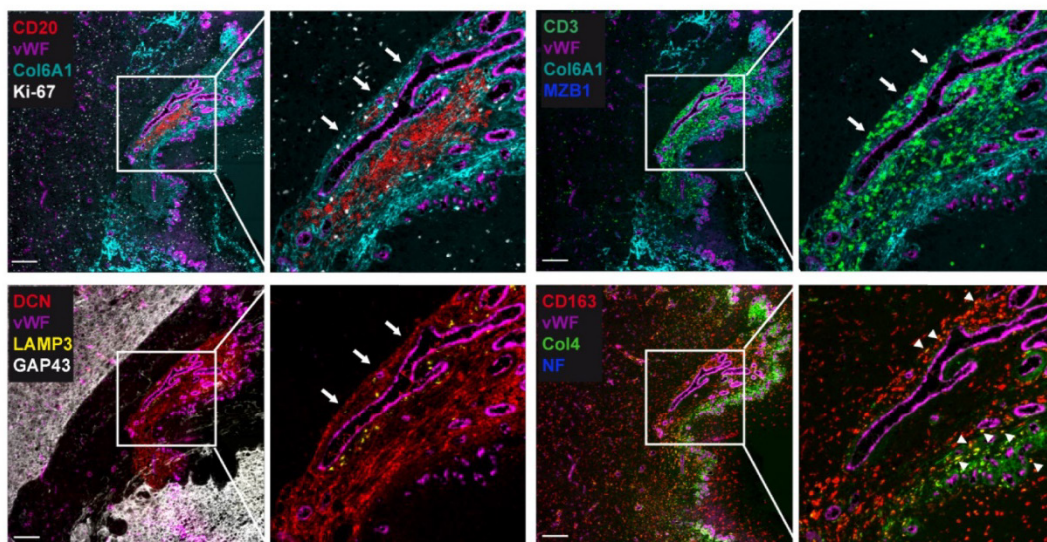
Example 3: Multiplex immunofluorescence for identification of colorectal cancer biomarkers



Mono- and pan-chromatic mIHC profile of CRC tissue^[3].

4

Example 4: Multiplex immunohistochemistry for spatial profiling of glioma subsets with functional tertiary lymphoid structures



TLS localized within a dense Col6A1 ECM surrounding a vWF blood vessel^[4].

Commonly Used Analysis Software

Supported by mainstream analytical software platforms such as HALO, Oncotopix, HistoCAT and QuPath, TSA can accurately identify the types and expression levels of target antigens in tissue samples, and precisely localize their distribution at the cellular and subcellular levels. Meanwhile, standardized quantitative analysis enables the acquisition of accurate antigen expression values. Furthermore, it allows in-depth exploration of the spatial proximity and functional interaction patterns among different antigens. This enables researchers and clinical practitioners to obtain multi-dimensional and three-dimensional biomolecular information from a single tissue section.

Software Name	Software Attribute	Core Functional Features	Advantages	Limitations
HALO	Commercial paid quantitative digital pathology platform	Image registration, tissue/cell segmentation, protein quantification, batch TMA analysis, multiplex fluorescence combined analysis, abundant plug-ins	Compatible with most digital slide formats, adapted to multiplex fluorescence imaging systems with comprehensive functions	Mainstream analytical platform in the industry
Oncotopix	Commercial paid AI-powered digital pathology software	AI-driven lesion identification, biomarker quantification, automated report generation, LIS connectivity	Certified for IVD clinical use, excellent performance in tumor heterogeneity analysis	High licensing cost, limited flexibility for customized research workflows
HistoCAT	Free open-source high-dimensional tissue analysis tool	Single-cell phenotyping, cellular spatial interaction analysis, microenvironment network analysis	Zero cost, specialized in cell neighborhood interaction research	Native segmentation performance is inadequate; preprocessing with auxiliary software is required
QuPath	Free open-source whole-slide high-throughput analysis software	Lossless analysis of oversized slides, batch processing, script-based customized analysis	No restriction on image size, ideal for large-cohort research	Limited accuracy of automated scoring for heterogeneously distributed complex biomarkers

Contrast with Traditional Methods

From Limitations to Breakthroughs — Key Differences in Sensitivity, Multiplexing, and Sample Preservation.

Traditional methods

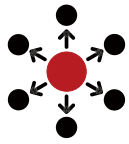
Conventional methods rely on enzymatic chromogenic reaction or direct fluorescent labeling, where signal intensity depends on the intrinsic abundance of target proteins and antibody binding efficiency. They are mostly applied for single-target detection and only support the simultaneous analysis of a limited number of biomarkers.

TSA

TSA adopts HRP-mediated enzymatic cascade signal amplification combined with multiple rounds of cyclic staining. It not only significantly enhances signal intensity, but also enables high-throughput in situ detection of multiple targets on a single tissue section. With distinct advantages in detection sensitivity and multi-biomarker parallel analysis, TSA stands out as an advanced and superior alternative to conventional pathological detection approaches.

	EnkiLife TSA kits	Traditional methods
Sensitivity	High	low
Number of single slice marks	3-7 color multi-markers	2-3 types
The degree of damage to the sample	Gently eluting and protecting tissues	The damage is large and needs to be repaired at high temperatures
Antibody species limit	Compatible with rabbit/mouse primary antibodies with no species restrictions	Strict
Fluorescence stability	It can be stored at 4°C for 12 months	It is easy to quench
Difficulty of operation	Ready-to-use	Complex

Core Advantages



7-Plex Signals from a Single Slide

7 spectrally resolved TSA fluorophores (480-750 nm) for any experimental panel.



Flexible Antibody Selection

Compatible with primary antibodies from rabbit and mouse.



Broad Applications

Works with FFPE sections, frozen sections, cells, and organoids.



All-in-One Components

Complete kit with tyramides, buffers, reagents, and mounting medium.



Gentle Antibody Elution

Special buffer removes antibodies while preserving antigens and tissue integrity.



Product Overview

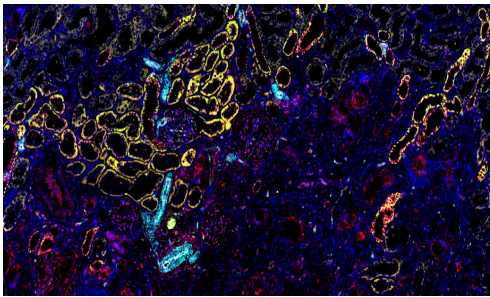
Composition and specifications

RA10012 7-Color Multiple Fluorescent Staining Kit

Components	Components			Dilutions
	20T	50T	100T	
TSA-CY3	10μL	20μL	50μL	1:200
TSA-FITC	10μL	20μL	50μL	1:200
TSA-680	10μL	20μL	50μL	1:200
TSA-430	10μL	20μL	50μL	1:200
TSA-594	10μL	20μL	50μL	1:200
TSA-CY7	10μL	20μL	50μL	1:200
TSA diluent	12mL	30mL	60mL	Ready-to-use
3% H ₂ O ₂	2mL	5mL	10mL	Ready-to-use
Blocking solution	12mL	30mL	60mL	Ready-to-use
Primary antibody diluent	12mL	30mL	60mL	Ready-to-use
Antibody elution reagent	10mL	25mL	50mL	Ready-to-use
HRP-goat anti-rabbit/mouse IgG	12mL	30mL	60mL	Ready-to-use
DAPI staining	2mL	5mL	10mL	Ready-to-use
Anti-fluorescence fading mounting medium	2mL	5mL	10mL	Ready-to-use

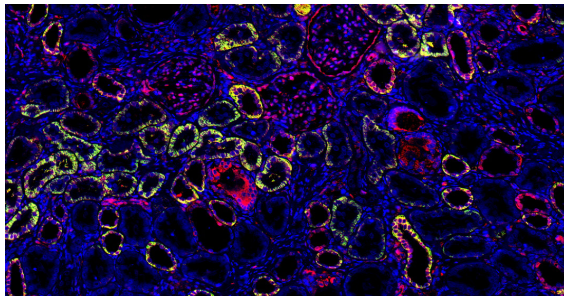
Illustrative Images

Test Sample: Rat kidney



AQP4 : Positive staining is evident in principal cells of the inner-medullary collecting ducts as well as in cortical and outer-medullary collecting ducts
CD31 : Widely used for labeling vascular endothelium; expressed in glomerular capillary endothelium and vessels at all hierarchical levels
TGF β 1 : Expression is markedly up-regulated in hypertension and fibrosis models; detected in glomerular epithelial cells (primarily podocytes) and interstitial fibroblasts
Zo-1 : As a scaffolding protein of tight junctions, it preserves the glomerular filtration barrier and tubular epithelial polarity
Collagen I : In fibrotic or sclerotic models, it extensively accumulates in the interstitium and glomeruli

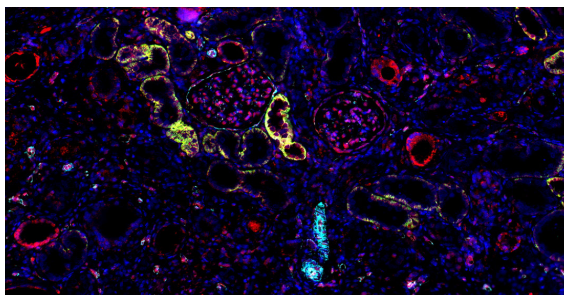
Target	Fluorescein	Ex (nm)	Em (nm)	Color	Filters	Image Color
AQP4	TSA-430	430nm	480nm		Aqua channel	Yellow
CD31	TSA-FITC	490nm	520nm		FITC channel	Green
TGF β 1	TSA-CY3	550nm	570nm		CY3 channel	Red
Zo-1	TSA-594	590nm	620nm		Red channel	Cyan
Collagen I	TSA-680	680nm	700nm		CY5/CY5.5 channel	Purple



3-Color Multiple Fluorescent Staining Kit (mIHC)

Cat. No.: RA10008

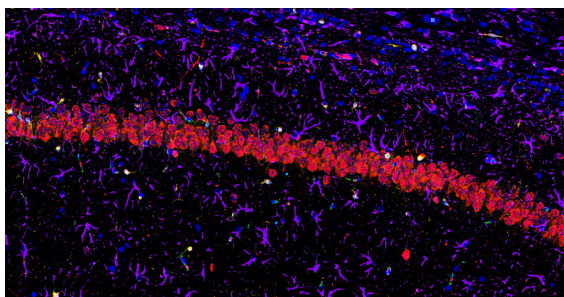
Size: 20T, 50T, 100T



4-Color Multiple Fluorescent Staining Kit (mIHC)

Cat. No.: RA10009

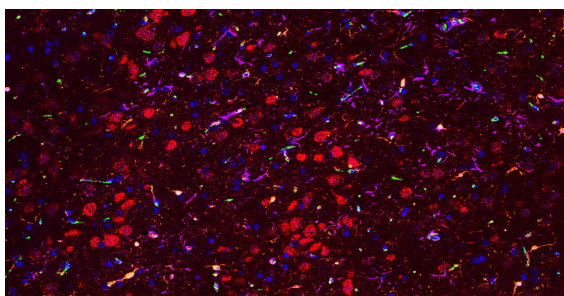
Size: 20T, 50T, 100T



5-Color Multiple Fluorescent Staining Kit (mIHC)

Cat. No.: RA10010

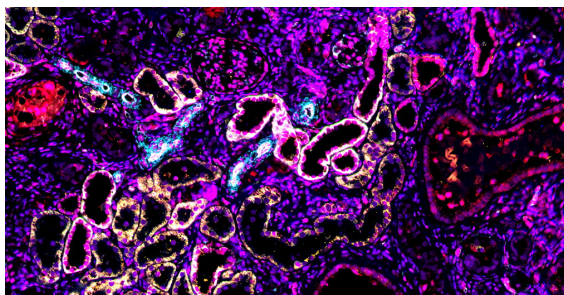
Size: 20T, 50T, 100T



6-Color Multiple Fluorescent Staining Kit (mIHC)

Cat. No.: RA10011

Size: 20T, 50T, 100T



7-Color Multiple Fluorescent Staining Kit (mIHC)

Cat. No.: RA10012

Size: 20T, 50T, 100T

Common FAQs

Q: Can frozen floating sections be used for TSA experiments?

A: TSA kits are not applicable to frozen floating sections. These sections are excessively thick, which impedes adequate reagent penetration. Additionally, floating sections are prone to morphological alterations such as curling and wrinkling during the staining process.

Q: How to select antibodies for mIHC of paraffin sections?

A: Monoclonal antibodies are recommended. Antibodies validated for IHC / mIHC / IHC-P applications should be selected, and knockout-validated antibodies are preferred.

Q: What causes non-specific binding?

A: ① Polyclonal antibodies easily cause non-specific binding. This problem can be improved by switching to monoclonal antibodies. ② Excessively strong signal amplification can also lead to background; reduce the incubation time or working concentration of the staining solution. ③ Overly high primary antibody concentration or excessive antigen retrieval will induce non-specific staining. It is recommended to use a higher dilution ratio of primary antibody or appropriately reduce the antigen retrieval strength.

Q: How to prevent section detachment?

A: ① Slides without adhesive coating are prone to falling off. Use positively charged adhesion slides or perform anti-drop coating treatment on slides. ② Incomplete tissue fixation or insufficient dehydration will affect tissue adhesion. Optimize sample pre-treatment by prolonging fixation and dehydration time. ③ Overly harsh thermal antigen retrieval may cause detachment. Adopt mild microwave retrieval, control the temperature at about 80 °C or lower, and use 2× citrate buffer (pH 6.0). ④ Too high-temperature of elution buffer may lead to tissue shedding; lower the buffer temperature or shorten the incubation time.

Q: What causes fluorescence crosstalk?

A: ① It may be related to the filter bandwidth of the imaging device; narrow-bandwidth filters are recommended. ② Residual antibodies from the previous round are not completely eluted. For high-affinity antibodies such as CK, strengthen elution

conditions by increasing elution temperature and incubation duration. ③ Unbalanced signal intensity between adjacent channels. Excessively strong fluorescence signals may overflow into adjacent channels and cause crosstalk. ④ Other operational errors include mixed use of antibodies or omission of antibody elution and antigen retrieval between staining rounds.

Q: For mouse samples, can primary antibodies of mouse origin be used?

A: It is generally not recommended. If the primary antibody is derived from mouse, the corresponding secondary antibody will also be anti-mouse, which may cross-react with endogenous IgG in mouse tissue and result in non-specific binding. If it is necessary to use primary antibodies from the same species as the sample, a negative control test is recommended: incubate the sample only with secondary antibody without adding primary antibody, to verify whether non-specific background signals occur.

Q: How to arrange the order of targets and antibodies in multiplex staining?

A: Antibodies that are difficult to elute should be placed in the final round to avoid crosstalk. Alternate the application of rabbit and mouse primary antibodies. Difficult-to-stain biomarkers are recommended to be arranged in the early rounds. Low-expression targets should be paired with high-brightness and anti-photobleaching fluorophores and tested in early rounds. High-expression targets should use fluorophores with moderate intensity to prevent signal saturation and blurred histological details. For two co-expressed proteins, select fluorophores with a large emission wavelength difference.

Q: Can conventional fluorescence staining be combined with TSA multiplex immunofluorescence?

A: Yes, but conventional fluorescence staining is suggested to be arranged in the last round. During the TSA antibody elution process, the bound complex of primary and secondary antibodies will be completely washed away.

Q: Is light avoidance required during operation? How long can stained slides be stored?

A: The fluorescent dyes of TSA kit have excellent anti-photobleaching properties. No light avoidance is needed under indoor fluorescent light, and dark environment operation is not required. However, direct strong sunlight exposure to the kit should be avoided. Stained slides can be stored for 6 to 12 months at 4 °C.

References

- [1] Peng H, Wu X, Zhong R, Yu T, Cai X, Liu J, Wen Y, Ao Y, Chen J, Li Y, He M, Li C, Zheng H, Chen Y, Pan Z, He J, Liang W. Profiling Tumor Immune Microenvironment of Non-Small Cell Lung Cancer Using Multiplex Immunofluorescence. *Front Immunol.* 2021 Nov 4;12:750046. doi: 10.3389/fimmu.2021.750046. PMID: 34804034; PMCID: PMC8600321.
- [2] Mori H, Bolen J, Schuetter L, Massion P, Hoyt CC, VandenBerg S, Esserman L, Borowsky AD, Campbell MJ. Characterizing the Tumor Immune Microenvironment with Tyramide-Based Multiplex Immunofluorescence. *J Mammary Gland Biol Neoplasia.* 2020 Dec;25(4):417-432. doi: 10.1007/s10911-021-09479-2. Epub 2021 Feb 15. PMID: 33590360; PMCID: PMC7960613.
- [3] Zhang W, Song ZJ, Zhang BY, Wang JL, Guo Q, Sun ZW, Tang H. Multiplex immunohistochemistry indicates biomarkers in colorectal cancer. *Neoplasma.* 2021 Nov;68(6):1272-1282. doi: 10.4149/neo_2021_210312N324. Epub 2021 Aug 30. PMID: 34459208.
- [4] Cakmak P, Lun JH, Singh A, Macas J, Schupp J, Schuck J, Mahmoud Z, Köhler M, Starzetz T, Burger MC, Steidl E, Hasse LM, Hattingen E, Plate KH, Reiss Y, Imkeller K. Spatial immune profiling defines a subset of human gliomas with functional tertiary lymphoid structures. *Immunity.* 2025 Nov 11;58(11):2847-2863.e8. doi: 10.1016/j.immuni.2025.09.018. Epub 2025 Oct 21. PMID: 41125076.

EnkiLife Related Products

Cat. No.	Product Name
RA10002	Antibody Dilution Buffer
RA10015	Antibody Diluent for IHC
PA10005	Endogenous Peroxidase Blocking Reagent
PA10006	Citrate Antigen Retrieval Buffer (20x, pH 6.0)
PA10007	Tris-EDTA Antigen Retrieval Buffer (20x, pH 9.0)
PA10012	Tris-EDTA Antigen Retrieval Buffer (10x, pH 9.0)
PA10013	One-step Dewaxing/Antigen Retrieval Buffer (20x, pH 6.0)
PA10018	One-step Dewaxing/Antigen Retrieval Buffer (20x, pH 9.0)
PA10019	Citrate Antigen Retrieval Buffer (10x, pH 6.0)
RA10006	HRP Anti-Mouse/Rabbit IHC Detection System(DAB solution)
RA10007	Polymer-HRP Anti-Mouse/Rabbit IHC Detection System(DAB solution)
PA10020	Rapid Ultra-Sensitive IHC Kit (pH 6.0)
PA10021	Rapid Ultra-Sensitive IHC Kit (pH 9.0)

Thank you
for choosing
us!