For Research Use Only

Five color TSA Detect Kit for Rabbit/Mouse Primary Antibody

Catalog Number: PK10034

www.ptglab.com

Product Information

TSA (Tyramide Signal Amplification) dyes are fluorescent labelling reagents based on TSA technology. When catalyzed by HRP, TSA dyes form covalent bonds with tyrosine residues on proteins near HRP and accumulate, thereby amplifying the signal. Compared to indirect detection using directly labeled fluorescent primary antibodies or fluorescent secondary antibodies, the TSA system can enhance the signal by several hundred-fold. Furthermore, the covalent bonds formed with the sample are stable and do not easily break, making this technique useful for experiments such as multiplex immunofluorescence and in situ hybridization.
This kit provides enough material to perform 5-color staining of 50 tissue sections or cell slides. It contains four different TSA dyes, nuclear DAPI stain, a Poly-HRP-Goat Anti-Rabbit/Mouse Universal Secondary antibody, amplification buffer, blocking/dilution buffer, and quenching buffer.

Components

Component	Volume	Concentration
Quenching Buffer	30 mL	RTU
Blocking/Dilution Buffer	100 mL	RTU
Multi-rAb™ Polymer HRP-Goat Anti-Rabbit/Mouse Universal Recombinant Secondary Antibody (H+L)	20 mL	RTU
CoraLite®Plus 488-Tyramide Reagent	100 uL	50X
CoraLite®Plus 555-Tyramide Reagent	100 uL	50X
CoraLite®Plus 594-Tyramide Reagent	100 uL	50X
CoraLite®Plus 647-Tyramide Reagent	100 uL	50X
Amplification Buffer	30 mL	RTU
DAPI Nuclear Stain	5 mL	RTU

This product allows for universal staining of mouse and rabbit primary antibodies. If you plan to only use rabbit primary antibodies, we suggest you use our 5 color TSA Detect Kit for Rabbit Primary Antibodies (PK10035).
The fluorescence excitation and emission spectra of the provided dyes are as follows, please use an appropriately configured imaging device:

· **CoraLite®Plus 488 -Tyramide Reagent**, maximum excitation wavelength is 493 nm, maximum emission wavelength is 522 nm.

· **CoraLite®Plus 555 -Tyramide Reagent**, maximum excitation wavelength is 555 nm, maximum emission wavelength is 570 nm.

· **CoraLite®Plus 594 -Tyramide Reagent**, maximum excitation wavelength is 588 nm, maximum emission wavelength is 604 nm.

· **CoraLite®Plus 647-Tyramide Reagent**, maximum excitation wavelength is 654 nm, maximum emission wavelength is 674 nm.

· **DAPI Nuclear Stain**, maximum excitation wavelength is 358 nm, maximum emission wavelength is 461 nm.

As the CoraLite®Plus 555-Tyramide and CoraLite®Plus 594-Tyramide excitation and emission wavelengths are relatively close, it is recommended to use these dyes with lower abundance targets or weaker primary antibodies. When imaging, use a narrow band pass filter to avoid signal streaks.

Package

50 T

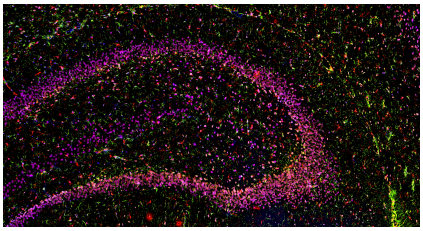
Storage

Store at 2-8°C for up to 12 months.

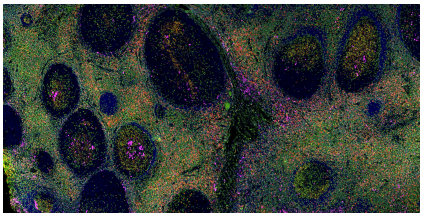
Notes

1. If performing multiplexed staining, it is generally recommended to stain low-abundance targets or weak antibodies first. For example, when using Proteintech TSA dyes, the recommended color matching order is: 555-488-594-647. Also, since CoraLite®Plus 555-TSA and CoraLite®Plus 594-TSA have similar wavelengths and are prone to bleed-through, it is recommended that CoraLite®Plus 555-TSA and CoraLite®Plus 594-TSA used with low abundance targets.
2. If performing multiplexed staining, it is recommended to first perform single staining to explore the optimal conditions for different targets before performing multiple rounds of staining to reduce unnecessary waste and facilitate analysis of results.
3. During the operation, when adding liquid to the sample at any step, ensure that there are no bubbles in the liquid, otherwise abnormal staining such as plaques will form. When adding liquid, be careful not to scratch the tissue.
4. Pay attention to the tissue sections or cell samples during the experiment and prevent them from drying out.

Validation Data



IF staining using PK10034 (Five color TSA Detect Kit for Rabbit/Mouse Primary Antibody). FFPE-rat brain tissue was stained with Lamin B1 monoclonal antibody (Cat.NO. 66095-1-Ig) at 1:2000 followed by detection with Multi-rAb® Polymer HRP-Goat Anti-Rabbit/Mouse Universal Recombinant Secondary Antibody (H+L). CoraLite®Plus 555-... Tyramide Reagent was used for signal development (yellow). The slide was then conducted HIER in pH 9.0 Tris-EDTA buffer followed by staining with GFAP monoclonal antibody (60190-1-Ig, 1:20000). CoraLite®Plus 488-Tyramide Reagent were used for signal development (green). Similarly, IBA1 recombinant antibody (81728-1-RR, 1:2000) TDP-43 recombinant antibody (80002-1-RR, 1:3200) were stained subsequently. CoraLite®Plus 594-Tyramide Reagent (red) and CoraLite®Plus 647-Tyramide Reagent (purple) were used for signal development respectively. Finally, cell nucleus (blue) were stained with DAPI. The signal from each channel was captured and merged.



IF staining using using PK10034 (Five color TSA Detect Kit for Rabbit/Mouse Primary Antibody). FFPE-human tonsillitis tissue was stained with CD23 monoclonal antibody (60208-2-Ig) at 1:8000 followed by detection with Multi-rAb® Polymer HRP-Goat Anti-Rabbit/Mouse Universal Recombinant Secondary Antibody (H+L).... CoraLite®Plus 555-Tyramide Reagent was used for signal development (yellow). The slide was then conducted HIER in pH 9.0 Tris-EDTA buffer followed by staining with CD3 monoclonal antibody (60181-1-Ig, 1:10000). CoraLite®Plus 488-Tyramide Reagent were used for signal development (green). Similarly, CD8a monoclonal antibody (66868-1-Ig, 1:20000) and CD138 monoclonal antibody (67155-1-Ig, 1:10000) were stained subsequently. CoraLite®Plus 594-Tyramide Reagent (red) and CoraLite®Plus 647-Tyramide Reagent (purple) were used for signal development respectively. Finally, cell nucleus (blue) were stained with DAPI. The signal from each channel was captured and merged.