Tyramide Lumo Kit

Table 1 Contents and storage

Component	Single Fluorescence (#TLK0301)	3-Plex (#TLK0601)	5-Plex (#TLK0603)	Concentration	Storage
HRP Blocking Buffer	10 mL	20 mL	40 mL	1×	2 to 8 °C
Antibody Dilution & Blocking Buffer	10 mL	20 mL	40 mL	1×	2 to 8 °C
Tyramide Lumo Buffer	10 mL	20 mL	40 mL	1×	2 to 8 °C
HRP polymer-conjugated Secondary Antibody (Anti-Mouse/Rabbit)	5 mL	10 mL	20 mL	N/A	2 to 8 °C
Tyramide Dye	50 µL per tube (100 slides) or 100 µL per tube (200 slides)			200×	2 to 8 °C for 6 months; −20 °C up to 12 months.
DAPI	10 mL			1×	2 to 8 °C

Introduction

Tyramide Signal Amplification (TSA) is a highly sensitive technology used to enhance the signal in varied immunocytochemistry (ICC) or immunohistochemistry (IHC) experiment for the detection of low-abundance targets. The detection sensitivity of TSA is 50-100 times greater than that of conventional ICC/IHC methods. Tyramide Lumo Kit combines the high-performance MaxFlour and MaxSulf fluorescent dyes with the TSA technology to make your ICC/IHC experiment more sensitive, more precise, and also easier.

Principles of TSA

The horseradish peroxidase-linked (HRP-linked) secondary antibody activates the tyramide fluorescent dye, which can then covalently label the target recognized by the primary antibody to create a high-density fluorescent area *in situ*, achieving the sensitive detection of the low-abundance targets. With TSA technology, multiple fluorescent dyes could be used to examine various targets in one sample simultaneously, as the primary antibody can be removed without affecting the covalently bound fluorescence.

Multiplex TSA is compatible with various types of ICC and IHC experiments and can be adapted to many other applications, including fluorescent *in-situ* hybridization (FISH).

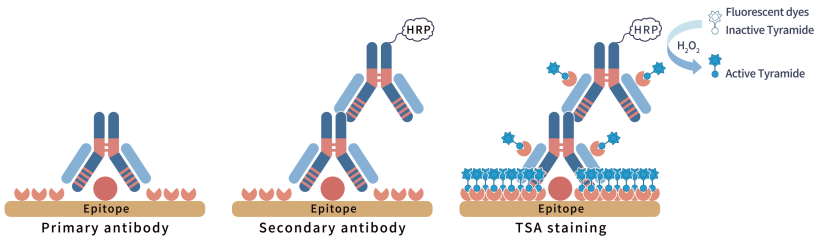

Figure 1 | Principles of Tyramide Signal Amplification

Before You Begin

Please prepare the following reagents which are not supplied in the kit:

Table 2 Materials not provided

PBS buffer	Xylene	Ethanol
Citrate Sodium Buffer (pH 6.0)	Paraformaldehyde	Methanol
Tris-EDTA Buffer (pH 9.0)	Triton X-100	Primary antibody
AntiFade Mounting Medium		

Preparing Reagents

Tyramide dye

The Tyramide Lumo Kit offers tyramide dye in two different concentration: 200× and 1×. The 200× Tyramide dye should be diluted with the provided Tyramide Lumo Buffer at a ratio of 1:50 to 1:200 before use. For targets with extremely low abundance, we recommend increasing the concentration of the dye. For lyophilized tyramide dye, please dissolve the dye by adding 100 µL of DMSO to the tube, resulting in a final concentration of 200×. Approximately 50 to 100 µL of the diluted tyramide dye is needed for each slide.

Table 3 Tyramide dye dilution scheme (1:200)

Material	Number of coverslips (24 × 24 mm^2)				
	5	10	20	50	100
Tyramide Lumo Buffer	500 µL	1 mL	2 mL	5 mL	10 mL
Tyramide Dye (200×)	2.5 µL	5 µL	10 µL	25 µL	50 µL

Preparing primary antibody

Dilute the primary antibody with the provided Antibody Dilution & Blocking Buffer according to the manufacturer's instructions. Prepare 50 to 100 µL of the diluted primary antibody for each slide.

Table 4 Preparing primary antibody

Material	Dilution ratio				
	1:50	1:100	1:500	1:1000	1:2000
Antibody Dilution & Blocking Buffer	1 mL	1 mL	1 mL	1 mL	1 mL
Primary Antibody	20 µL	10 µL	2 µL	1 µL	0.5 µL

Methods

Preparing cell coverslips

1. Fixation Fix the cells on the coverslip for 5–10 minutes in 100% methanol or 4% paraformaldehyde at 4 °C. Gently rinse the fixed cells with PBS for 5 minutes to remove any fixation agent. Repeat rinse step two more times.

2. Permeabilization Incubate the coverslips in 0.3%–0.5% Triton X-100 in PBS at room temperature for 5–10 minutes. Wash the coverslips with PBS for 5 minutes to remove the permeabilization agent. Repeat this wash step two more times.

Preparing paraffin-embedded tissue sections

3. Deparaffinization Soak the tissue section in 100% xylene for 10 minutes to remove paraffin. Replace the xylene with fresh 100% xylene and soak the tissue section for an additional 10 minutes.

4. Hydration Soak the tissue section in 100% ethanol for 10 minutes. Replace the ethanol with fresh 100% ethanol and soak for an additional 10 minutes. Then, sequentially soak the tissue section in solution of 95%, 85%, 70%, and 50% ethanol for 5 minutes each, followed by washing with ddH_2O for 5 minutes.

5. Antigen retrieval Soak the tissue section in citrate sodium buffer (pH 6.0) or Tris-EDTA (pH 9.0). Boil the solution using a microwave heater at the maximum power, then continue heating the boiled solution at 20% of maximum power for 15 minutes. After cooling down to room temperature, rinse the tissue section with ddH_2O for 5 minutes, followed by rinse three times with PBS for 5 minutes each.

Quenching endogenous peroxidase (optional)

6. If needed, quench the endogenous peroxidase activity by adding HRP Blocking Buffer to the sample and incubating at room temperature for 10–20 minutes. Then wash the sample thoroughly with PBS.

Blocking non-specific binding sites

7. Add 2–3 drops (about 100–150 µL) of Antibody Dilution & Blocking Buffer to the sample and incubate at room temperature for 30 minutes.

Peroxidase labelling

8. Incubate the cells or tissue with a dilute mouse or rabbit primary antibody at room temperature for 60 minutes. Alternatively, incubation could be performed overnight at 2–8 °C. Wash the cells or tissue three times with PBS for 5–10 minutes each.

9. Add 2–3 drops (about 100–150 µL) of HRP polymer-conjugated Secondary Antibody (Anti-Mouse/Rabbit) to the sample and incubate at room temperature for 40–60 minutes. After incubation, wash the cells or tissue with PBS three times for 5–10 minutes each.

TSA staining

10. Add 100 µL of 1× tyramide dye to cover the sample and incubate at room temperature for 5–15 minutes. The final concentration of the dye and the incubation time could be adjusted according to the expression level of the targets of interest.

Multiplex TSA staining

11. TSA staining can be multiplexed with spectrally compatible tyramide dyes. Perform cell permeabilization or antigen retrieval again to remove previously labelled antibodies, followed by peroxidase labelling and TSA staining as described in step 8 to 10.

DAPI staining (optional)

12. Add 2–3 drops (about 100–150 µL) of DAPI (4',6-diamidino-2-phenylindole) to cover the sample and incubate in the dark at room temperature for 10 minutes. Wash the sample three times with PBS for 5 minutes each.

Mounting slides

13. Mount the slides using antifade mounting medium following the manufacturer's instructions. Avoid the formation of bubbles in the mounting medium.

Microscopy and imaging

14. Analyze the cells or tissue with fluorescent microscope or other compatible imaging systems.

Appendix I: Signal optimization

To acquire the most specific and high-resolution results, we highly recommend optimizing experimental conditions, including dilution ratio of the antibody, concentration of tyramide dye, and staining time.

Primary antibody amount

The amount of primary antibody applied greatly affects the result. We recommend pre-testing of different dilution ratios of the primary antibody and including positive and negative slides to determine the optimal dilution ratio.

Tyramide dye dilution ratio

The 200× tyramide dye should be diluted using the provided Tyramide Lumo Buffer before use. The optimal dilution ratio ranges from 1:50 to 1:200. Consider using tyramide dye of a higher concentration for extremely low-abundance targets, and vice versa.

Staining time

TSA staining time is another crucial factor affecting the specificity and resolution of the result. Staining time of 2.5, 5, 7.5, 10, and 15 minutes can be tested with positive and negative slides. If the signal is dim, increase the staining time. If non-specific signal presents in negative controls or blurry fluorescence presents in positive controls, decrease the staining time.

Appendix II: Troubleshooting

Table 5 Troubleshooting

Issue	Solution
Excess signal	Shorten TSA staining time
	Decrease the amount of primary antibody and shorten incubation time
Low signal	Increase TSA staining time
	Increase the amount of primary antibody and extend incubation time
	Change antigen retrieval buffer
High background	Change cell fixation solution
	Quench the endogenous HRP acitivity for a longer time
	Extend the blocking time for non-specific binding sites
	Rinse samples thoroughly in corresponding steps, or rinse samples with PBST buffer
	Decrease the amount of secondary antibody or shorten incubation time

Appendix III: Order information

Table 6 Signal Fluorescence

Series	Product	Cat#	Size	Ex/Em (nm)	Color
MaxFluor Tyramide Lumo Kit（1x）	MaxFluor 485 Tyramide Lumo Kit	TLK0101	50 slides	439/485	
	MaxFluor 525 Tyramide Lumo Kit	TLK0102	50 slides	490/525	
	MaxFluor 555 Tyramide Lumo Kit	TLK0103	50 slides	534/555	
	MaxFluor 605 Tyramide Lumo Kit	TLK0104	50 slides	578/605	
	MaxFluor 675 Tyramide Lumo Kit	TLK0105	50 slides	650/675	
	MaxFluor 705 Tyramide Lumo Kit	TLK0106	50 slides	679/705	
	MaxFluor 715 Tyramide Lumo Kit	TLK0107	50 slides	691/715	
MaxSulf Tyramide Lumo Kit（1x）	MaxSulf 565 Tyramide Lumo Kit	TLK0201	50 slides	554/565	
	MaxSulf 665 Tyramide Lumo Kit	TLK0202	50 slides	647/665	
	MaxSulf 775 Tyramide Lumo Kit	TLK0203	50 slides	756/775	
MaxFluor Tyramide Lumo Kit（200x）	MaxFluor 485 Tyramide Lumo Kit	TLK0301	100/200 slides	439/485	
	MaxFluor 525 Tyramide Lumo Kit	TLK0302	100/200 slides	490/525	
	MaxFluor 555 Tyramide Lumo Kit	TLK0303	100/200 slides	534/555	
	MaxFluor 605 Tyramide Lumo Kit	TLK0304	100/200 slides	578/605	
	MaxFluor 675 Tyramide Lumo Kit	TLK0305	100/200 slides	650/675	
	MaxFluor 705 Tyramide Lumo Kit	TLK0306	100/200 slides	679/705	
	MaxFluor 715 Tyramide Lumo Kit	TLK0307	100/200 slides	691/715	
MaxSulf Tyramide Lumo Kit（200x）	MaxSulf 565 Tyramide Lumo Kit	TLK0401	100/200 slides	554/565	
	MaxSulf 665 Tyramide Lumo Kit	TLK0402	100/200 slides	647/665	
	MaxSulf 775 Tyramide Lumo Kit	TLK0403	100/200 slides	756/775	

Table 7 Multiplex Fluorescence

Series	Product	Cat#	Size	Color
Tyramide Lumo Kit（1x）	3-Plex Tyramide Lumo Kit	TLK0501	50 slides	
	4-Plex Tyramide Lumo Kit	TLK0502	50 slides	
	5-Plex Tyramide Lumo Kit	TLK0503	50 slides	
	6-Plex Tyramide Lumo Kit	TLK0504	50 slides	
	7-Plex Tyramide Lumo Kit	TLK0505	50 slides	
Tyramide Lumo Kit（200x）	3-Plex Tyramide Lumo Kit	TLK0601	100/200 slides	
	4-Plex Tyramide Lumo Kit	TLK0602	100/200 slides	
	5-Plex Tyramide Lumo Kit	TLK0603	100/200 slides	
	6-Plex Tyramide Lumo Kit	TLK0604	100/200 slides	
	7-Plex Tyramide Lumo Kit	TLK0605	100/200 slides	

Table 8 Tyramide Dye

Series	Product	Cat#	Size	Ex/Em (nm)	Color
MaxFluor Tyramide（Lyophilized）	MaxFluor 485 Tyramide	FLD0101	200 slides	439/485	
	MaxFluor 525 Tyramide	FLD0102	200 slides	490/525	
	MaxFluor 555 Tyramide	FLD0103	200 slides	534/555	
	MaxFluor 605 Tyramide	FLD0104	200 slides	578/605	
	MaxFluor 675 Tyramide	FLD0105	200 slides	650/675	
	MaxFluor 705 Tyramide	FLD0106	200 slides	679/705	
	MaxFluor 715 Tyramide	FLD0107	200 slides	691/715	
MaxSulf Tyramide（Lyophilized）	MaxSulf 565 Tyramide	FLD0201	200 slides	554/565	
	MaxSulf 665 Tyramide	FLD0202	200 slides	647/665	
	MaxSulf 775 Tyramide	FLD0203	200 slides	756/775	

For more product information, please visit: **www.msbiox.com**