# Product Information

## YF®350/488/555/594/640/680/Biotin Tyramide Kit

Product Size: 50 slides, 200 slides

Catalog Number:

| Catalog Number | Product Name |
|---|---|
| Y6081 | YF®350 Tyramide Kit, HRP Goat anti-mouse IgG |
| Y6082 | YF®350 Tyramide Kit, HRP Goat anti-rabbit IgG |
| Y6083 | YF®350 Tyramide Kit, HRP Streptavidin |
| Y6084 | YF®488 Tyramide Kit, HRP Goat anti-mouse IgG |
| Y6085 | YF®488 Tyramide Kit, HRP Goat anti-rabbit IgG |
| Y6086 | YF®488 Tyramide Kit, HRP Streptavidin |
| Y6087 | YF®555 Tyramide Kit, HRP Goat anti-mouse IgG |
| Y6088 | YF®555 Tyramide Kit, HRP Goat anti-rabbit IgG |
| Y6089 | YF®555 Tyramide Kit, HRP Streptavidin |
| Y6090 | YF®594 Tyramide Kit, HRP Goat anti-mouse IgG |
| Y6091 | YF®594 Tyramide Kit, HRP Goat anti-rabbit IgG |
| Y6092 | YF®594 Tyramide Kit, HRP Streptavidin |
| Y6093 | YF®640 Tyramide Kit, HRP Goat anti-mouse IgG |
| Y6094 | YF®640 Tyramide Kit, HRP Goat anti-rabbit IgG |
| Y6095 | YF®640 Tyramide Kit, HRP Streptavidin |
| Y6096 | YF®680 Tyramide Kit, HRP Goat anti-mouse IgG |
| Y6097 | YF®680 Tyramide Kit, HRP Goat anti-rabbit IgG |
| Y6098 | YF®680 Tyramide Kit, HRP Streptavidin |
| B6099 | Biotin Tyramide Kit, HRP Goat anti-mouse IgG |
| B6100 | Biotin Tyramide Kit, HRP Goat anti-rabbit IgG |
| B6101 | Biotin Tyramide Kit, HRP Streptavidin |



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Contents:

**Table 1: Name, Number and Volume of components**

| Number | Name of component | Volume | |
|---|---|---|---|
| | | 50 slides | 200 slides |
| In Table 2 | Tyramide Stock Solution, 200× | 25 μL | 100 μL |
| In Table 2 | HRP-conjugated secondary antibody | 25 μL | 100 μL |
| 60011 | BSA | 0.5 g | 2 g |
| 60012 | 1× Tyramide Amplification Buffer | 5 mL | 20 mL |

**Table 2: Number of components in the kit**

| Label Dyes | Ex/Em | HRP-conjugated secondary antibody | | | YF®/Biotin Tyramide |
|---|---|---|---|---|---|
| | | Goat anti-mouse IgG | Goat anti-rabbit IgG | Streptavidin | |
| YF®350 | 347/448 | 60008 | 60009 | 60010 | 60001 |
| YF®488 | 490/515 | 60008 | 60009 | 60010 | 60002 |
| YF®555 | 555/565 | 60008 | 60009 | 60010 | 60003 |
| YF®594 | 593/614 | 60008 | 60009 | 60010 | 60004 |
| YF®640 | 642/662 | 60008 | 60009 | 60010 | 60005 |
| YF®680 | 681/698 | 60008 | 60009 | 60010 | 60006 |
| Biotin | ~ | 60008 | 60009 | 60010 | 60007 |

## Storage

Store at -20℃ and protect from light. When stored as directed, product is stable for at least 6 months.

## Description

TSA (tyramide signal amplification), also known as CSA (catalyzed signal amplification), is a kind of enzyme detection method that uses HRP to carry out high-density in situ labeling of target antigens. It can not only be used for IF / IHC signal amplification, but also for ELISA, ISH and other detection methods.

TSA technology can be used to detect low abundance targets which can not be detected by traditional methods. The signal amplification technology based on tyramide can provide strong sensitivity and detect very small amount of target antigen. TSA technology greatly reduces the dosage of antibody. TSA kit can be used in combination with traditional staining methods for polychromatic imaging, two or more tyramide reactions can be performed sequentially to mark different targets on a sample, too.

## Protocol

**Self prepared reagent:**

- 1 × PBS
- Paraformaldehyde fixative （ 4% Paraformaldehyde in PBS）
- Osmogenic agent（0.5% Triton X-100 in PBS）
- 0.1 M Sodium citrate buffer（pH 6.0）



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- 30% Hydrogen Peroxide
- Blocking buffer: Dissolve 1g BSA in 100 mL PBS containing 0.5% Triton X-100 to prepare blocking buffer. You can also choose blocking buffer sold on the market.

The following components are only used in Biotin-Tyramide Kit:

- Biotin blocking wash buffer:1% BSA and 0.05% Tween 20 in PBS.
- Unlabeled streptavidin solution: 0.1 mg/mL streptavidin in biotin blocking wash buffer.
- Biotin solution: 0.5 mg/mL biotin in biotin blocking wash buffer.

The following reference procedure can be used for tyramide staining of cell or tissue sections, using 100 µL of dye solution per sample (sufficient to cover one well of 96 well plate or about 1 $cm^2$ of tissue part). The volume can be increased or decreased according to different sample sizes.

**1. Sample preparation**

1.1 Cell sample

1). Optional: prepare a negative control sample (incubate without primary antibody).

2). Wash cells twice with 1 × PBS.

3). Cell fixation: add appropriate 4% paraformaldehyde (pH 7.4) solution, and place at 4 ℃ for 15 min.

4). Wash cells twice with 1 × PBS.

5). Cell permeabilization: cells can be permeated with 0.5% Triton X-100 in PBS and placed at room temperature for 10 min. Or add 70% precooled ethanol and incubate at -20 ℃ for 4 h. Cells can be preserved for one week at -20 ℃ in 70% ethanol.

6). Wash cells twice with 1 × PBS.

1.2 Paraffin section

1). Place paraffin sections in an oven at 60 ℃ for 30 minutes.

2). Paraffin sections were immersed in dimethylbenzene twice at room temperature for 5 min each time to remove paraffin thoroughly.

Note: dimethylbenzene is toxic and volatile. Please operate in the fume hood.

3). At room temperature, the slices were immersed in absolute ethanol and rinsed twice for 5 min each time.

4). At room temperature, the section samples were immersed in ethanol (95%, 90%, 80%, 70%) of different concentration gradients, and each concentration was rinsed once for 5 minutes.

5). Immerse the slice in pure water for 3 minutes at room temperature, and then immerse the slice in 1 × PBS for 3 minutes. Carefully suck the excess liquid around the slice sample with filter paper.

6). The contour of the sample was drawn around the section sample with an immunohistochemical pen so that it could be penetrated and labeled downstream.

7). Antigen repair: heat 0.1M citrate buffer solution (pH = 6.0) to boiling with microwave oven, put the dewaxed and rehydrated tablets in the buffer solution, and boil for 10 minutes intermittently. Note that during this process, the tissue on the slide should always be immersed in the buffer to ensure the antigen repair effect of the tissue. After the duration of antigen repair, take it out and let it cool down slowly at room temperature.

Note: different methods of antigen repair should be selected according to different samples.

8). Wash samples twice with 1 × PBS.

**2. (Optional) endogenous peroxidase inactivation**

If necessary, the endogenous peroxidase activity of the sample was quenched by adding enough 3% hydrogen peroxide to cover the sample and incubation at room temperature for 60 min.

**3. (Optional) endogenous biotin blocking**

For streptavidin / biotin detection, we recommend blocking endogenous biotin in the sample to reduce the background. This

step can be omitted for kits labeled with YF®-Tyramide and HRP-conjugated secondary antibody.

1). At room temperature, incubate samples with unmarked streptavidin solution for 15 minutes, and then washed at room temperature for 3 times with biotin blocking wash buffer for 5 minutes each time.

2). Incubate samples with biotin solution for 30 min at room temperature to block the excess biotin binding sites on streptavidin. After that, wash samples with biotin blocking wash buffer for 3 times, each time for 5 minutes.

**4. Immunolabelling**

1). Block: block for 1h at room temperature with blocking buffer. Generally, blocking buffer sold on the market needs 10 minutes.

2). Dilute primary antibody to an appropriate concentration with blocking buffer. Incubate sample with primary antibody at room temperature for 1 h or 4 ℃ overnight.

Note: if the kit is used with streptavidin, please use biotin-conjugated primary antibody.

3). Wash 3 times with 1 × PBS at room temperature for 5 min each time.

4). Dilute HRP-conjugated secondary antibody with blocking buffer at 1:200 . Incubate at room temperature for 1 h.

5). Wash 3 times with 1 × PBS at room temperature for 5 min each time.

6). The dye solution was prepared by diluting Tyramide Stock Solution with 1 × Tyramide Amplification Buffer at 1:200. Prepare 100 μL dye solution for each sample. The dye solution can be stored for 24 hours at room temperature in dark.

7). Incubate the sample with the dye solution at room temperature for 10 min.

8). Wash 3 times with 1 × PBS at room temperature for 5 min each time.

9). **Optional:** if you choose Biotin-Tyramide Kit, fluorescent-streptavidin can be used for visualization, or you can use streptavidin-HRP with DAB.

10). Microscopic imaging. For tissue samples on slides, cover the slides and seal them before microscopic imaging.

## Notes

1. After 1 × Tyramide Amplification Buffer is used for the first time, it is recommended to pack it separately in small quantity and store at - 20℃ to avoid repeated freezing and thawing.

2. Compared with fluorescent-secondary antibody, Tyramide Kit showed higher sensitivity and stronger signal. Therefore, the concentration of the primary antibody is low in this experiment, which can reduce the background fluorescence caused by non-specific binding. We suggest setting concentration gradient of the primary antibody to get the optimal one.

3. If you need to take the background fluorescence into consideration, it is recommended to set a negative control without incubate with primary antibody. Ensure that the negative control is not cross contaminated by reagents in positive samples during incubation and washing. For tissue samples, we also recommend imaging an unstained control (without adding antibodies or tyramide) to find out the effect of tissue spontaneous fluorescence on the background.

4. It is recommended to use 5 μg / mL HRP conjuncts, reducing the concentration may affect the signal strength and sensitivity.

5. It is recommended to dilute YF® / Biotin-Tyramide at 1:200. Higher concentrations may lead to too strong signal or high background. You can explore from 1:100 to 1:1000 to find the optimal concentration.

6. After each tyramide labeling reaction, different targets on one sample can be labeled successively by HRP quenching or antibody stripping using multiple Tyramide Kits.



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