

# **Tyramide Signal Amplification (TSA)**

This tyramide signal amplification (TSA) protocol can be used to detect signals in peroxidase-labeled samples by either immunostaining or *in situ* hybridization. All incubations are carried out on a shaker.

## **Reagents:**

- 2-4% formaldehyde, pH 7.4
- phosphate buffer (PBS), pH 7.4
- PBT: 0.1% Tween-20; PBS, pH 7.4
- 1mM sodium azide in PBT
- 30% H<sub>2</sub>O<sub>2</sub>
- 1 mg/ml fluorescently labeled tyramide in labeling grade DMSO

### Optionally:

- dextran sulfate (DS)
- 4-iodophenol
- acidic glycine buffer: 0.1 M glycine; pH 2.0; 0.1% Tween-20

# **Protocol:**

- 1. Fix the sample in the required manner. Usually, fixation is carried out with cold 2-4% formaldehyde (pH 7.4). Wash the sample from the fixative with phosphate buffer (PBS, pH 7.4) and **PBT** (0.1% Tween-20; PBS, pH 7.4).
- Inhibit endogenous peroxidase activity by incubating the sample in an **inhibitory solution** (1 mM sodium azide in PBT) for 30-60 min at room temperature.

*Optionally.* Instead of 1 mM sodium azide,  $3\% H_2O_2$  in PBT or 0.02 N HCl can be used for inhibition. For subsequent immunochemistry, this stage can be combined with blocking nonspecific binding of antibodies. To do this, blocking serum or 1% BSA must be added to the azide or  $H_2O_2$  solutions.

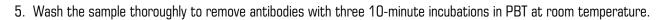
*Note!* In the case of low background and for *in situ* hybridization, this step can be skipped; go directly to step 4.

- 3. Thoroughly wash the sample from the inhibitory solution with three 10-minute incubations in PBT at room temperature.
- 4. Perform all immunochemistry or *in situ* hybridization steps to label samples with peroxidase-conjugated antibodies.

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6. Prepare **reaction buffer** immediately before use. To do this, dilute PBT twice with  $30\% H_2O_2$ , 1:100 each time, to the final concentration of 0.003% (1:10000).

*Optionally.* To increase the sensitivity of the method, the following components can be added to the reaction mixture (separately or in combination):

- $\circ$  2% dextran sulfate (DS) is used to increase the viscosity of the reaction mixture.
- 500 µg/ml 4-iodophenol is used to enhance the peroxidase reaction. It is more convenient to use as a 1:200 dilution of the stock solution (100 mg/ml in ethanol).
- 7. Add 1 to 10  $\mu$ g/ml labeled tyramide to the reaction buffer (the optimal concentration must be determined experimentally). Mix by shaking.
- 8. Incubate the sample with the reaction buffer in the dark at room temperature for 5–30 min (the exact time is determined experimentally; 15 min can be used as a starting point).
- 9. Stop the reaction by incubating the sample in inhibitor solution (1 mM sodium azide in PBT) for 10 min at room temperature in the dark.
- 10. Wash the sample with PBS three times for 10 min.

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- 11. To repeat the antibody-TSA labeling cycle, treat the sample with **acidic glycine buffer** (0.1% Tween-20; 0.1 M glycine, pH 2.0) for 10 min at room temperature. This procedure detaches antigen-bound antibodies without affecting the tyramide covalently bound to proteins.
- 12. Mount the sample under a coverslip using a mounting medium.

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# Troubleshooting

PROBLEM	RECOMMENDED ACTION
Low signal	<ul> <li>Optimize probe / antibody concentration</li> <li>Titer HRP conjugate to determine optimum concentration for signal amplification</li> <li>Add tissue permeabilization step to facilitate penetration of reagents</li> <li>Lengthen the incubation time with the tyramide working solution</li> <li>Use antigen retrieval techniques to unmask the target epitopes</li> </ul>
Excess signal	<ul> <li>Optimize probe / antibody concentration</li> <li>Decrease concentration of HRP conjugate</li> <li>Decrease the tyramide concentration in working solution</li> <li>Shorten the incubation time with the tyramide working solution</li> </ul>
Low resolution or blurry signal	<ul> <li>Shorten the incubation time with the tyramide working solution</li> <li>Check the dilution of the stop reagent</li> </ul>
High background	<ul> <li>Decrease probe / primary antibody concentration</li> <li>Use a lower concentration of secondary antibody</li> <li>Decrease HRP conjugate concentration</li> <li>Check for endogenous biotin (if using streptavidin conjugates)</li> <li>Check for antibody specificity</li> <li>Shorten the incubation time with the tyramide working solution</li> <li>Lengthen endogenous peroxidase quenching step</li> <li>Increase number and/or length of washes</li> <li>Nonqualified or contaminated blocking reagent used</li> <li>Filter buffers</li> </ul>
Bright dots on background	<ul> <li>Centrifuge secondary antibody tube before use</li> </ul>

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