ABP Biosciences

405 E Gude Dr, STE 214
Rockville, MD 20850, USA
Web: www.abpbio.com
Email: info@abpbio.com

Andy Fluor™ Tyramide Conjugates

Table 1. Products and Storage

Cat. No.	Product Name	Ex/Em (nm)	Unit	Storage	Stability
L040	Andy Fluor 488 Tyramide	495/520	0.5 µmol	-20 °C	The product is stable for one year when stored as directed.
L041	Andy Fluor 555 Tyramide	553/565	0.5 µmol	-20 °C	
L042	Andy Fluor 594 Tyramide	590/615	0.5 µmol	-20 °C	
L043	Andy Fluor 647 Tyramide	650/666	0.5 µmol	-20 °C	

Introduction

Andy Fluor™ tyramide conjugates are used for tyramide signal amplification (TSA), which is a highly sensitive method enabling the detection of low-abundance targets in fluorescent immunocytochemistry (ICC), immunohistochemistry (IHC), and *in situ* hybridization (FISH) applications.

TSA uses horseradish peroxidase (HRP) to generate high-density labeling of a target protein or nucleic acid *in situ*. The target is labeled with HRP-conjugated detection reagent (e.g., antibody or streptavidin). The HRP-labeled sample is then incubated with labeled tyramide and hydrogen peroxide, which converts the labeled tyramide substrate into a highly reactive form. Multiple reactive tyramide molecules conjugate to tyrosine residues in the target to generate high density tyramide labeling. This leads to significant amplification of the signal at the target and is the reason for the exceptional sensitivity of this system.

Multiple TSA procedures can be performed sequentially to label different targets on the same sample by performing HRP quenching or antibody stripping after each tyramide reaction. The label that is covalently attached to the sample will remain.

Considerations for Tyramide Signal Amplification

TSA reactions using HRP-conjugated antibodies typically use tyramide concentrations of 1-5 µM in a reaction buffer containing 0.0015% hydrogen peroxide.

For best results, we recommend diluting tyramides in reaction buffer just before use. However, tyramides can be diluted in reaction buffer up to 24 hours before staining for use with automated staining instruments.

General Protocols

1. Dissolve the tyramide in 1 mL DMSO to make 0.5 mM stock solution. The stock solution can be stored at -20 °C for one year.
2. Fix, permeabilize, and block cell or tissue samples following general immunohistochemistry protocols.

   Note: Inactivation of endogenous peroxidase activity may be required for some tissues or cell types.
3. Incubate samples with blocking buffer containing 10 mg/mL BSA and 0.5% Triton® X-100 for 1 hour at room temperature.

4. Dilute the primary antibodies using blocking buffer according to the manufacturer's guidelines. Incubate samples with primary antibodies at room temperature for 1 hour or 4°C overnight. Wash 3 × 5 minutes with PBS.
5. Incubate samples with 5 µg/mL HRP conjugate in blocking buffer at room temperature for 1 hour. Wash 3 × 5 minutes with PBS.

   Note: HRP conjugates must be diluted in azide-free buffer.
6. Prepare a tyramide working solution using 10 mM Tris buffer, pH 7.4 or other commercially available reaction buffer. Add hydrogen peroxide at a final concentration of 0.0015%. Dilute the tyramide conjugate in the working solution at a final concentration of 5 µM.
7. Apply 100 µL of the tyramide working solution to the cells or tissue and incubate for 10 minutes at room temperature, then rinse 3 times with PBS.
8. Mount samples with mounting medium and image fluorescence using the appropriate excitation and emission settings.