Tyramide Signal Amplification (TSA) Detection

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

DAPI (4', 6' –diamidino-2-phenylindole hydrochloride)
Sigma, Cat. 18860
Dimethyl sulfoxide (DMSO)
Sigma, Cat. D-2650
Ethyl alcohol (200 proof), anhydrous
Formamide
Fluka BioChemika, Cat. 47671
Hydrogen Peroxide
Methyl alcohol, anhydrous
Mallinckrodt AR (ACS), Cat. 3016
Phosphate Buffered Saline (PBS) 1X
Sodium Chloride (NaCl), Crystal
J.T. Baker, Cat. 3624-01
SSC, 20X
Tris/HCl , 1 M (pH 8.0)
Tween 20 (Polyoxyethylene–sorbitan monolaurate)
Sigma, Cat. P1379
Tyramide (TSA) -Indirect (ISH) Kit
TSA™ Biotin System, for 200-600 slides
Perkin Elmer, Cat. NEL 700001KT (technical support: perkinelmer.com)
Kit includes:

- **Streptavidin-HRP (Horseradish peroxidase) Conjugate**
- **DuPont Blocking Reagent**
- **Amplification Diluent**
- **Biotinyl-tyramide (Tyr)**
- Store kit at 4°C

Preparation

50% Formamide/2X SSC
20X SSC 30 ml
dH_2O 120 ml
Formamide 150 ml
Adjust pH to 7.5 with 1 N HCl
Pre-warm to 45˚C

0.1X SSC
20 X SSC 2.5 ml
dH_2O 497.5 ml
Pre-warm to 60˚C

TNT Buffer
1 M Tris/HCl, pH 8.0 100 ml
5 M NaCl 30 ml
Tween20 1 ml
dH_2O 869 ml
Adjust pH to 7.5, RT.

Blocking Solution (0.5% in TNT buffer)
Blocking reagent 0.05 g
TNT buffer 10 ml
To dissolve the blocking reagent, heat 0.5% blocking reagent solution to 60°C for 1 hr with stirring. Blocking reagent may be stored up to one month at -20°C.
Pre-warm to 37˚C

Biotinyl Tyramide Working Solution
Biotinyl tyramide (BT) is supplied as a solid which needs to be reconstituted in DMSO. Use 1.2 ml DMSO for the 200-600 kit and 0.3 ml for the smaller kit. This stock solution is stable for 6 months when stored at 4°C. Before each procedure, make a 1:50 dilution of the BT Stock solution using the Amplification Diluent. Approximately 300 µl of BT Working Solution is required per slide.

DAPI stock solution (f.c.= 0.2 mg/ml)
DAPI 2 mg
ddH_2O 10 ml
Aliquot and store at -80ºC

DAPI staining solution (f.c.= 80 ng/ml)
DAPI (stock solution) 40 µl
2X SSC 100 ml
Store at 4˚C in a light-tight coplin jar

Procedure

1. Following hybridization of the indirect-labeled probe, remove rubber cement and coverslips gently, and wash slides in 50% formamide/2X SSC 3 x 5 min each at 45°C.

2. Wash slides in 0.1X SSC 3 x 5 min each at 60°C.

3. Wash slides in TNT Buffer once, 5 min, while shaking.

4. Add 120 µl per slide of blocking reagent, cover with 24 mm x 60 mm coverslip, incubate in a humidity chamber for 30 min at RT (see note 5).

5. Add 120 µl per slide of Strep-Avidin~HRP, using a 1:100 dilution; the antibody is dissolved in 0.5% blocking solution. Cover slide with coverslip, incubate slides in light-tight humidity chamber for 30 min at RT.

6. Wash slides 3 x 5 min each in TNT Buffer , while shaking. Keep slides in a light-tight coplin jar.

7. Shake off excess buffer, apply 300 µl tyramide solution (1:50 dilution), per slide, incubate slides in a light-tight humidity chamber without a coverslip for 5 min at RT.

8. Drain off tyramide solution by tapping slide onto paper towel.

9. Wash 3 x 1 min with TNT buffer at RT.

10. To detect Tyr~bio, use fluorochrome-conjugated Avidin (example, Avidin-FITC) diluted 1:200 in 0.5% blocking solution and incubate for 45 min at 37°C in humidity chamber.

11. Wash with TNT buffer 3 x 5 min each at RT.

12. Counterstain slides with DAPI staining solution for 5 min at RT.

13. Wash slides in 2X SSC for 5 min, while shaking.

14. Dehydrate slides in ethanol series 70%-90%-100% ethanol 2 min each; air dry.

15. Apply 35 µl antifade to each slide and cover with 24 mm x 60 mm coverslip (see **Antifade** under **FISH** protocols)

Notes

1. Tyramide Signal Amplification (TSA) is an extremely powerful signal amplification system that boosts hybridization signals up to 1000 fold. It can be used for both fluorescence and chromogenic in situ hybridizations using standard immunological techniques. The term “indirect” is used to indicate that after amplification, additional steps are needed to give a detectable signal, i.e., the deposited biotin of TSA-Indirect requires additional standard enzymes such as fluorescent streptavidin conjugates.

2. Tyramide Signal Amplification is based upon the catalyzed reporter deposition technique using derivatized tyramide (a phenolic compound which reacts with electron rich moieties on a surface). Horseradish peroxidase (HRP) catalyzes the deposition of biotinyl tyramide onto tissue sections or cell preparation surfaces blocked with protein.

3. TSA can be applied for FISH mapping of biotin-labeled PCR products. The lower limit of resolution of DNA probes is approximately 300-500 bp.

4. Prior to hybridization, slides must be pre-treated according to the following protocol.

   a) Wash slides in a coplin jar containing 50 ml of 3% hydrogen peroxide (H_2O_2) in methanol for 10 min. This will quench exogenous peroxidases present on slides.

   b) Wash slides in coplin jar with shaking for 5 min with 50 ml 1X PBS.

   c) Equilibrate slides in 50 ml 2X SSC. Follow FISH pre-treatment protocol (see **FISH** Protocols).

5. Do not substitue other blocking reagents (BSA for example) for the one supplied by the kit, as you may increase the background significantly.

References

1. Bobrow MN & Litt GJ: Method for detection or quantitation of an analyte dependent enzyme activation system. US patent no 5196306, 1993.

2. Kerstens HMJ, et al: CARD-ISH signal amplification. J Histochem Cytochem 43:347, 1995.

3. Raap AK, et al: Ultra-sensitive FISH using peroxidase-mediated deposition of biotin- or fluorochrome tyramides. Hum Mol Genet 4:529, 1995.

4. Van Gijlswijk RPM, et al: Improved localization of fluorescent tyramides for fluorescence in situ hybridization using dextran sulphate and polyvinyl alcohol. J Histochem Cytochem 44:389, 1996.

5. Schriml LM, Padilla-Nash HM, Coleman A, Moen P, Nash WG, Menninger J, Jones G, Ried T, and Dean M: Tyramide Signal Amplification (TSA)-FISH applied to mapping PCR-labeled probes less than 1 kb in size. Biotechniques 27:608-613,1999.

Note: Reference is found on Riedlab/members/ as pdf file for Hesed Padilla-Nash.