NBT Staining

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Introduction

Modified from: Paz, M.A., Fluckiger, R., Boak, A., Kagan, H.M., and Gallop, P.M. (1991). Specific detection of quinoproteins by redox-cycling staining. ***J. Biol. Chem.*** *266,* 689-692.

Reagents

- Prepare a 2M potassium glycinate buffer at pH 10. Dissolve 75g glycine in 400mL ddH_2O, pH to 10 with KOH and bring to 500mL and store at 4ºC

- Prepare a 0.16M sodium borate solution. Add 2.5g sodium borate to 40mL ddH_2O and heat in microwave to dissolve.

- Immediately before use prepare a 0.6mg/mL NBT (Nitro blue tetrazolium, Sigma N-6876) solution in the potassium glycinate buffer. In an aluminum foil covered 15mL Falcon tube dissolve 8-9mg NBT in 14mL of the potassium glycinate buffer. Mix well, undissolved NBT will leave dark spots on the membrane.

Protocol for NBT Staining

1. Place Nylon membrane in small flat bottomed container (Petri dishes work well if using a membrane from a half gel).

2. Pour NBT solution over membrane and incubate at RT in the dark for 45 minutes.

3. Pour off NBT solution and wash membrane with sodium borate solution 2 times.

4. Soak membrane in sodium borate solution over night.

5. Wash membrane with ddH_2O

6. Membranes can be stored in ddH2O or dried between paper towels. Photograph before drying for best results.

Comments

A 0.5µL drop of 2.5µg/mL EGCG on nylon (0.4ng EGCG/mm^2) can be visualized if there were no brown spots from undissolved NBT.

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