BIOTIN DETECTION IN ELECTROPHORETIC GELS


1. Run gel with appropriate controls and molecular weight markers (e.g. Biotinylated DNA MW marker)

2. Block non-specific binding:
   a. Place the gel in a clean plastic or glass dish.
   b. Add 20 ml freshly prepared TBS (see below).
   c. Incubate on a platform rocker at room temperature for 30 minutes.

3. Bind streptavidin to the biotinylated proteins
   a. Add 10 µl streptavidin-alkaline phosphatase conjugate (Roche catalog # 1 089 161).
   b. Incubate on a platform rocker at room temperature for 30-120 minutes.

4. Detect alkaline phosphatase activity
   a. Decant streptavidin-alkaline phosphatase solution.
   b. Rinse gel briefly in 100 mM Tris pH 9.0. Decant.
   c. Add detection buffer.
   d. Incubate gel at 37ºC in the dark until bands appear.
   e. Rinse gel to remove detection buffer.

5. Photograph gel.
   For gel preservation, use protocol for drying at room temperature. Heat drying is not recommended, as the alkaline phosphatase activity could turn the whole gel blue.

TBS (Tris Buffered Saline)  
(make fresh each time)  
H₂O 45 ml  
gelatin powder 0.25 g

Heat to dissolve gelatin.
Cool to room temperature, then add:
1M Tris pH 8.0 5 ml  
5M NaCl 0.6 ml  
Make TBS fresh every time

Detection buffer  
1M Tris pH 9.0 10 ml  
H₂O 85 ml  
1 M MgCl₂ 200 µl  
1% X-P* 5 ml  
NBT* 10 mg

Mix thoroughly to ensure NBT is dissolved before adding to gel to prevent punctate staining.

*X-P = 5-bromo, 4-chloro, 3-indolyl phosphate, prepared in DMSO or DMF (50 mg in 5 ml)  
NBT= nitro blue tetrazolium

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