WARNING NOTICE: The experiments described in these materials are potentially hazardous and require a high level of safety training, special facilities and equipment, and supervision by appropriate individuals. You bear the sole responsibility, liability, and risk for the implementation of such safety procedures and measures. MIT shall have no responsibility, liability, or risk for the content or implementation of any of the material presented. Legal Notices

BIOTIN DETECTION IN ELECTROPHORETIC GELS

Ref: Gennady P. Manchenko (1994) Handbook of Detection of Enzymes on Electrophoretic Gels. CRC Press, Boca Raton. P. 187

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)

H_2O	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
H_2O	85 ml
1 M $MgCl_2$	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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