Detection of polyol dehydrogenases activity in native polyacrylamide gels

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Abstract

Polyol dehydrogenases are enzymes that convert polyalcohols into sugars, using NAD^+ as hydrogen acceptor. Sorbitol, mannitol or other polyalcohols can be used as substrates and NADH is produced. In this assay, proteins are first separated by native polyacrylamide gel electrophoresis and the gel is then incubated with the enzyme substrates. NADH produced by the dehydrogenase reduces tetranitro blue tetrazolium chloride (TNBT), generating a black product that stains the gel. This procedure gave good results with polyol dehydrogenases from the digestive gland of gastropods and, with the proper adaptations, can be applied to other dehydrogenases and other organisms.

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Before start

Prepare the following solutions:

Homogenization medium for marine gastropods

(adapted from Stewart et al. 1994 and Moyers et al. 1991)

Sucrose - 500 mM

Potassium chloride - 150 mM

Ethylenediamine tetraacetic acid (EDTA), sodium salt - 1 mM

Tris-HCl 50 mM pH 7.4

This medium, without phenylmethylsulfonyl fluoride (PMSF), can be stored at -20º C in aliquots. To avoid the inactivation of PMSF in *aqueous solution,* add 5 µl of 200 mM PMSF (in absolute ethanol) per ml of medium just before use (final PMSF concentration 1 mM).

Incubation Medium

(adapted from Lewis and Gibson, 1978).

Phenazine methosulphate - 0.01 mg . ml^{-1}

Tetranitro blue tetrazolium chloride (TNBT) - 0.1 mg . ml^{-1}

NAD^+ - 3 mg . ml^{-1} (» 4.5 mM)

Polyol substrate (mannitol, sorbitol or other polyol) - 50 mM

50 mM Tris-HCl, pH 8.0 (TNBT precipitates at higher pH)

Sample buffer for native gel electrophoresis

125 mM Tris-HCl, pH 6.8

80% glycerol

0.02% Bromophenol Blue

Materials

- Potter-Elvehjem homogenizer by Contributed by users
- Centrifuge by Contributed by users
- Ultracentrifuge by Contributed by users
- Gel electrophoresis equipment by Contributed by users
- 7.5% Polyacrylamide gel (precast or handmade) by Contributed by users

Protocol

Preparation of the cytosolic fraction

Step 1.

- Homogenize approximately 0.1 g of digestive gland tissue per ml of cold homogenization medium, using a Potter-Elvehjem homogenizer at 1,000 rpm, keeping the tube in ice.
- Centrifuge de homogenate at 15,000 g for 20 min at 4º C, and recover the supernatant.
- Centrifuge the previous supernatant at 100,000 g for 1 h at 4º C, recover the supernatant (cytosolic fraction) and store frozen in aliquots.
- Evaluated protein concentration in the cytosolic fraction, and adjust to 5 mg . ml^{-1} with buffer.

Detection of polyol dehydrogenases after native gel electrophoresis

Step 2.

- Mix 3 parts of the cytosolic fraction and 1 part of sample buffer.
- Load the samples on a 7.5% non-denaturant polyacrylamide gel and carry out the electrophoresis at 100 V during 4 h, at 4ºC.
- Incubate the gel for 3 h at room temperature in medium with substrate for detection of dehydrogenase activity. If necessary, incubation time can be extended.
- Use medium without substrate for incubation of a control gel.

References

Step 3.

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