Activity staining and Isozyme analysis

Requirements

1. Cheesecloth
2. Potato
3. All chemicals needed for SDS-PAGE except SDS
4. Sodium Chloride
5. Acid Phosphatase standard
6. 1-Naphthyl Phosphate
7. Fast blue RR
8. 10% MAGNESIUM CHLORIDE
9. 0.1m Acetate buffer

Methods

1. Polymerize acrylamide gel as explained in earlier experiments. As this is native gel don't use SDS in acrylamide, running buffer or sample buffers.

2. Peeled and diced potato tubers (100 g) were homogenized in 100 ml of 0.1M Acetate buffer (pH 5.0).The homogenate was squeezed through six layers of cheesecloth and centrifuged at 14,000g for 20 min. Supernatant fractions were pooled and designated the crude extract. Store a small fraction of crude extract in -20°C.
3. Load 10ul, 20ul and 30ul of crude extract with appropriate amount of sample buffer (as explained in SDS-PAGE experiment). In one or two lanes load 5-10ug (in 10ul volume) of standard Acid Phosphatase
4. Conduct the electrophoresis at low temperature (Preferably in cold room)
5. Wash the gel 3-4 times in 0.1M acetate buffer (pH 5.0) by changing the buffer every 15minutes (to lower the pH of the gel to 5.0). Incubate the gel at 37°C for 2-3 hours in following solutions.

1-Naphthyl Phosphate	0.05g
Fast blue RR	0.05g
Sodium Chloride	1g
10% Magnesium Chloride	0.5ml
0.1m Acetate buffer	50ml

Reading

De, K.K,. and Roy S.C. (1984) *Thero appl. Genetics.* 68, 285