

Enrichment of protein tyrosine kinase activity in yeast

Preparation of affinity column. 3 grams of CNBr-activated Sepharose 4B (Sigma) is incubated for 15 min. at room temperature with 30 ml of 1mM HCl. The swelled Sepharose is resuspended in 20 ml of coupling buffer (0.1M NaHCO₃, 0.5M NaCl, pH8.3) containing 150 mg of random synthetic copolymer of glutamate and tyrosine (4:1)(poly-E₄Y₁; molecular weight 20.000-60.000; Sigma) and mixed end-over-end overnight at 4°C. After centrifugation for 5 min. at 400 g, the poly-E₄Y₁-Sepharose suspension is resuspended in 20 ml of 0.2M glycine, pH 8.0 and mixed end-over-end overnight at 4°C. The suspension is centrifuged for 5 min. at 400 g, resuspended in 50 ml of coupling buffer, washed ten times with 50 ml of acid buffer (0.1M NaAc, 0.5M NaCl, pH 4.0), resuspended in 10 ml of coupling buffer and stored at 4°C.

Affinity purification of the protein tyrosine kinases. Cell lysates are prepared from 1 liter of an early stationary phase culture grown in YPD. Cells (5g wet weight) are washed twice with 20mM Tris-HCl pH 7.5, resuspended in 15ml of lysis buffer (20mM Tris-HCl pH 7.5, 1mM EDTA, 10 µg/ml leupeptin, 20µg/ml aprotinin, 2mM p-aminobenzamidine, 5µg/ml pepstatin), and passed twice through a French press at 15.000 pounds per square inch. The lysate is centrifuged at 10.000 g for 10 min. at 4°C to remove nuclei and cell debris. The supernatant is mixed with Triton X-100 (f.c. 1%), incubated for 30 min. on ice, and centrifuged at 100.000 g for 30 min. at 4°C. Resulting supernatant (10 ml) is combined with 5 ml of poly-E₄Y₁-Sepharose beads and mixed end-over-end for 60 min. at 4°C. Beads are collected in a column, washed with 25 ml of lysis buffer containing 0.1% Triton X-100 followed by 25 ml of the same buffer containing 0.1M NaCl. Bound proteins are eluted with linear gradient of 0.1-1.2M NaCl in lysis buffer containing 0.1% Triton X-100. Selected fractions are tested for the protein tyrosine kinase activity.

Reference:

Adamikova, L., Resnick, R.J. & Tomaska L. (1996) Enrichment of yeast protein tyrosine kinase activity by substrate affinity chromatography. *Yeast* 12: 833-838.