

Fluorescently Labeled Concanavalin A

- 8:30 1) Concanavalin A is dissolved in borate buffer, pH 9.6 containing Ca^{++} and Mn^{++} ions, 10^{-3}M - let stand 30 min
- Con A - 100 mg (weight)
Borate buffer - 0.02 M, pH 9.6 \pm Ca^{++} , Mn^{++} , 10^{-3}M - use 6 ml / 100 mg Con A
- 2) Dissolve α -D methyl glucose in ~~some~~ borate buffer
Dilute into 2 ml borate buffer
- 9:00 3) Add (2) to (1), mix & let stand for 1 hr. at room temp.
- 9:45 4) Mix FITC in borate buffer - 50 $\mu\text{g}/\text{mg}$ Con A = 500 μg = 5 mg FITC needed
(Dissolve well)
5 mg FITC into 2 ml borate buffer
- 10:00 5) SLOWLY add (4) to (3) over a 10 minute period while stirring at room temperature
- 12:00
5:00 6) Allow to stand at room temp without stirring for ~~(2)~~ hrs, then put in refrigerator until 5:00
i.e. 2 hrs room temp, 5 hrs at 4°C
- 9:00 change buffer 7) Dialyze against two 3 liter changes of Ca^{++} and Mn^{++} free Tris buffer, pH 7.4 for about 36 hours
Tris 0.01 M, pH 7.4
- 8) Separate active Con A from inactive Con A & Free Fluorescein
- a) Tris 0.01 M pH 7.4 containing $0.6 \times 10^{-3}\text{M}$ each of Ca^{++} & Mn^{++}
 - b) Mix 5 ml of (a) to 10 ml of dialyzed / fluorescent protein and let stand at 4°C for 1 hour
 - c) Put on G-100 column equilibrated with 0.01 M Tris, pH 7.4 (about 1 gm of Sepharose G-100 is enough for column)
 - d) Elute with Tris buffer until no more 250 (D) stuff comes off
 - e) Change to 0.02 M Glycine-HCl buffer pH 2.0 & elute active Con A
- (4) f) Neutralize with 0.05 M NaOH , dialyze overnight against PBS - 3 l. for