Furin Test Cleavage of GST-(IEGRG)-PAX(38-557)-FL Tuesday, Oct. 7, 2003

2 x Furin buffer made (40 mL total):
- 200 mM HEPES, pH 7.5 (phied w/ 5 N NaOH)
- 1% Triton X-100
- 2 mM CaCl2
- 2 mM 2-mercaptoethanol (mw = 78.13, d = 1.14 g/mL)

(above concentrations are for the 2 x solution)

NEB tech. services uses: 5 mg (= 70 pmol) protein in a 50 mL reaction and incubates with 1 or 2 units of Furin at 30°C for 1 to 2 hours for complete cleavage. Furin is active at 30°C for up to 48 hours.

A Furin cleavage rxn and a negative control were set up and incubated, first at 30 to 35°C for 40 min, then (because the water bath temp would not stabilize and I was nervous about overheating) at 24°C for the remainder of the reaction.

A: 30 mL (15 mg) of 0.5 mg/mL protein solution
   30 mL 2 x Furin buffer
   0 mL Furin

B: 30 mL (15 mg) of 0.5 mg/mL protein soin.
   30 mL 2 x Furin buffer
   1 mL (2 units) of Furin, 2000 units/mL

1  15 mL protein ladder
2  10 mL A, t = 0
3  10 mL B, t = 0
4  10 mL A, t = 1 hr
5  10 mL B, t = 1 hr
6  10 mL A, t = 2 hr
7  10 mL B, t = 2 hr
8  10 mL A, t = 4 hr
9  10 mL B, t = 4 hr

- The paxillin band does not seem to be degrading over time, but there is a ten...