

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

2 x Furin buffer made (40 mL total):

- 200 mM HEPES, pH 7.5 (pHed w/ 5 N NaOH)
  - 1% Triton X-100
  - 2 mM CaCl<sub>2</sub>
  - 2 mM 2-mercaptoethanol (mw = 78.13, d = 1.14 g/mL)
- (the above concentrations are for the 2x solution)

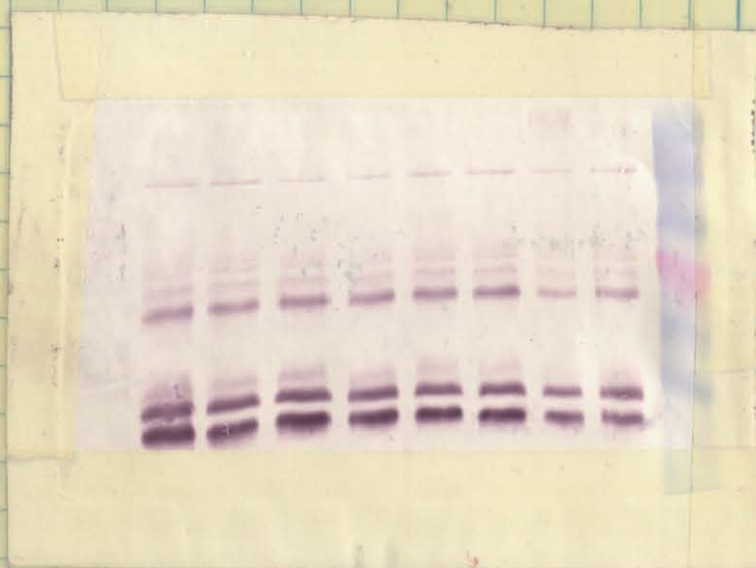
NEB tech. services uses: 5  $\mu$ g ( $\approx$  70 pmol) protein in a 50  $\mu$ L reaction and incubates with 1 or 2 units of Furin at 30°C for 1 to 2 hours for complete cleavage. Furin is active at 30°C for up to 48 hours.

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  $\mu$ L (15  $\mu$ g) of 0.5  $\mu$ g/mL protein solution  
 30  $\mu$ L 2x Furin buffer  
 0  $\mu$ L Furin

B: 30  $\mu$ L (15  $\mu$ g) of 0.5  $\mu$ g/mL protein soln.  
 30  $\mu$ L 2x Furin buffer  
 1  $\mu$ L (2 units) of Furin, 2000 units/mL

In 1 15  $\mu$ L protein ladder  
 2 10  $\mu$ L A, t = 0  
 3 10  $\mu$ L B, t = 0  
 4 10  $\mu$ L A, t = 1 hr  
 5 10  $\mu$ L B, t = 1 hr  
 6 10  $\mu$ L A, t = 2 hr  
 7 10  $\mu$ L B, t = 2 hr  
 8 10  $\mu$ L A, t = 4 hr  
 9 10  $\mu$ L B, t = 4 hr



9 8 7 6 5 4 3 2 1

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