

Phosphorylation of full-length Paxillin

Tuesday, June 28, 2005

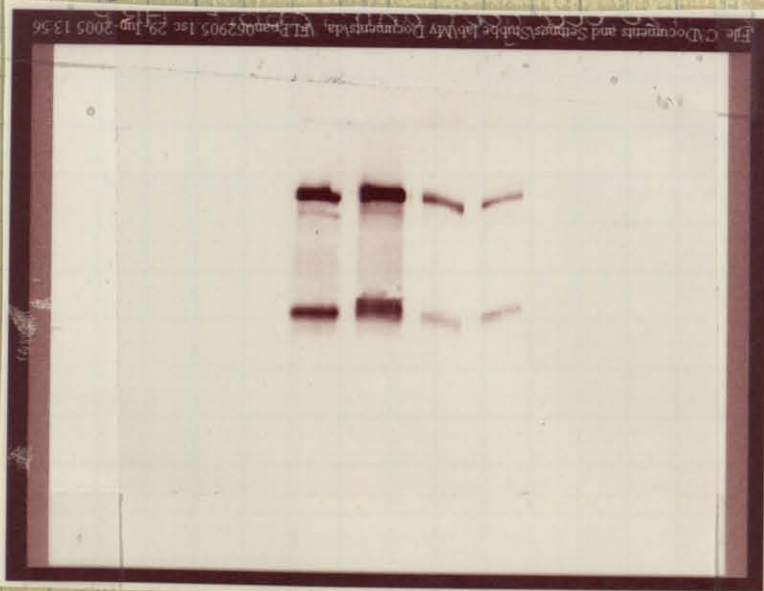
4 Reactions set up in 0.65 mL eppendorfs:

- 0.6 μg GST-Pax(1-557)-FLAG (2 μL of elution #2 on p. 126 at 300 $\mu\text{g}/\text{mL}$. Cells for prot. pur. had been lysed 6/23.)
~intended to use 2 μg of substrate~
- 4 μL of a 1 mM ATP solution (50 μM final conc.)
- 4.5 μL of a 226 mM MgCl_2 solution (15 mM final conc.)
- 0.8 μL of a 1 mM ZnCl_2 soln. (10 μM final conc.)
- 2 μL Src, or 2 μL JNK, or 4 μL ERK II, or no kinase
- (66.5 (or 64.5) μL of TBS

= 80 μL total

The above reagents were pipetted up and down 7 x and flicked once to mix, then incubated at 30°C for 15 min. The reactions were stopped w/ the addition of 80 μL 2x SDS reducing gel loading buffer (to give 3.75 ng/ μL) A 1/2 dilution of this was made with more loading buffer (1.9 ng/ μL).

4 gels were loaded w/ 4 μL (7.5 μg) / lane with 10 μL (19 ng) loaded for the ERK and JNK lanes. (intended to load 20 ng for Src / no enzyme and 50 ng for the others ~ less needed w/ the minimally-degraded substrate.



5 4 3 2 1

1° ab 1/10,000 mouse anti-paxillin

2° 1/10,000 goat anti-mouse HRP

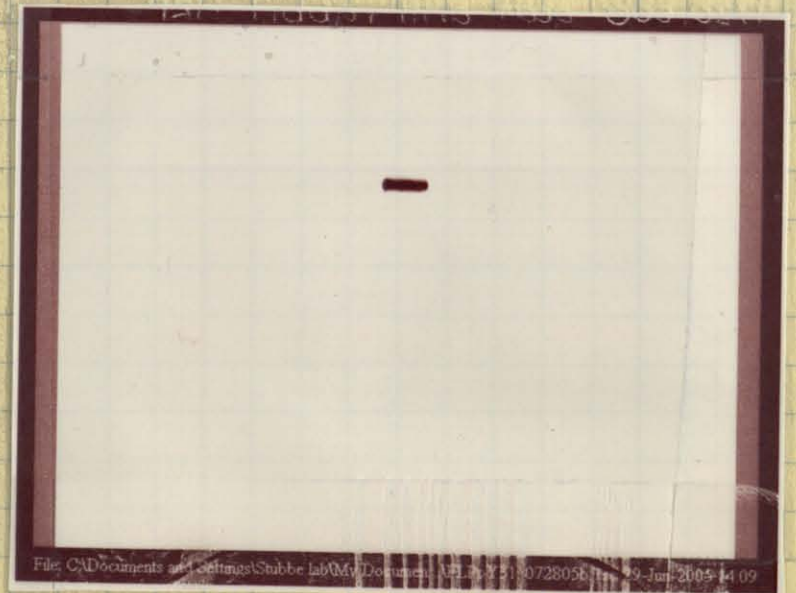
rxn with:

ln 2 7.5 ng no kinase

3 7.5 ng Src

4 19 ng ERK II

5 19 ng JNK



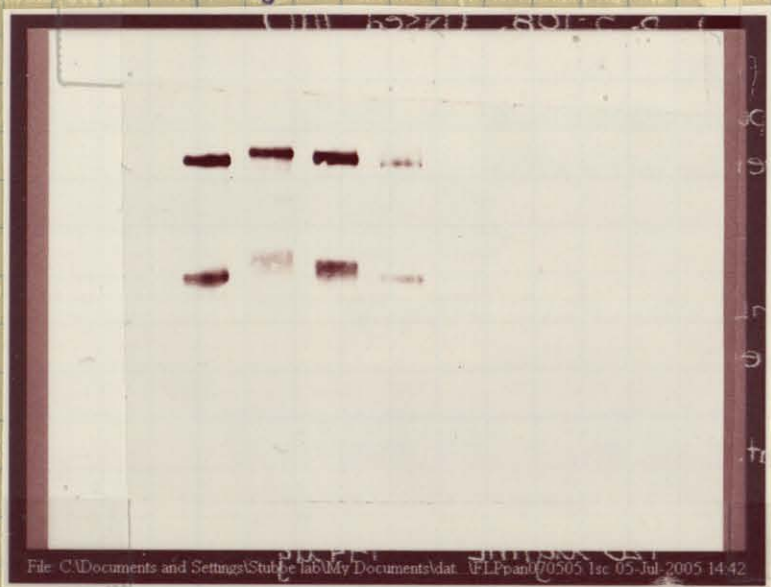
5 4 3 2 1

1° ab 1/20,000 rabbit anti-pY31 pax

2° 1/20,000 goat anti-rabbit HRP

Full-length Pax: GST-Pax(1-557)-FLAG

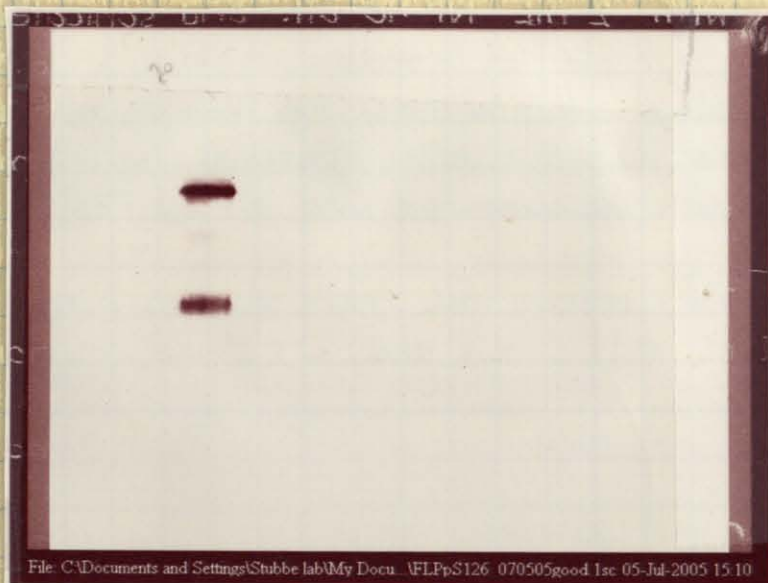
1° 1/1,000 mouse anti-paxillin
 2° 1/10,000 goat anti-mouse HRP



5 4 3 2 1
 20 ng/well loaded

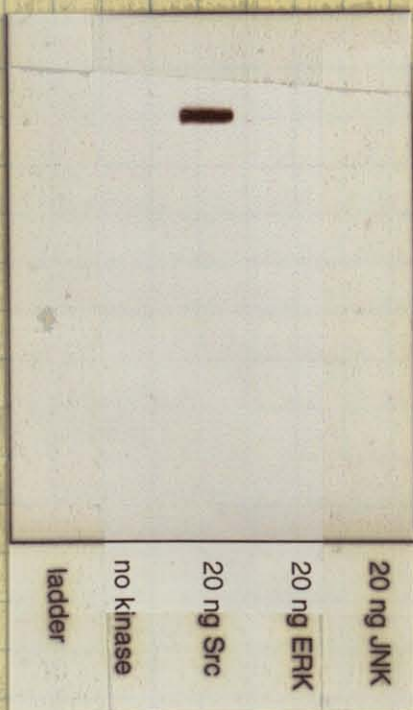
In 1 ladder
 2 ~~src rxn~~ no kinase
 3 src rxn.
 4 ERK rxn.
 5 JNK rxn

1° 1/2,000 rabbit anti-pS126
 2° 1/20,000 goat anti-rabbit HRP



5 4 3 2 1
 200 ng/well loaded

1° 1/2,000 rabbit anti-pY31
 2° 1/20,000 goat anti-mouse HRP



Summary of Full-length Pax phosphorylation
 (still confusing results w/ pS178...)

