GST pull down w/ FAK (857-1053) ~ no negative controls - just trying to get a positive readout as a starting point.

- GST-FAK (857-1053) elution #1 purified from Codon Plus RIL cells (p. 5-92 and 94) was exchanged into PBS (getting rid of the glutathione) by concentrating from 1 mL → 100 µL in a 10 kDa MWCO centrifugal filter (spun at < 3,000 g at 4°C). The protein was rediluted w/ PBS and then reconcentrated twice before taking up in a total of 1 mL PBS.

- 100 µL of fresh GST resin were incubated with the GST-FAK (857-1053) solution for 1 hr at room temperature. The flow through was collected (I'm expecting alot of FAK in the flow through b/c there should be a ton of FAK in the el. 1 and I wanted to bind all the GST resin.) The resin was washed with more than 4.0 mL of PBS.

- The resin was incubated for 2 hours at room temp. with His-purified AcHis6 Pax(2-36)-Cys-Pax(38-557)-FLAG (elutions #1 and 2 - 100 µL each - from p. 5-97.) The imidizole was not removed from the His elutions prior to incubation. The flow through was collected + resin washed w/ PBS.

- Two elution fractions were collected. For each, 200 µL of 10 mM reduced glutathione in 50 mM Tris HCl, pH 8.0 was incubated 5 min. with the resin and the Flow through was collected.

anti-FLAG 1°

1 2 3 4 5

anti-His6 ab

5 4 3 2 1

in 1 protein ladder
2 20 µL FAK flow thr. (10 µL per gel)
3 50 µL Paxillin flow through (25 µL per gel)
4 50 µL el. 1
5 50 µL el. 2

Yay! The pax can only be seen in the elutions, not in the Flow through. Although the GST-FAK has no FLAG or His tag, it is staining a bit with the ab, probably due to the sheer quantity of the protein.