

## Testing for Non-specific cleavage w/ enterokinase

BioRad enterokinase (from -20  $\leftarrow$  including buffer - not sure how old the kit is): 1 unit shown to cleave up to 50 mg test protein.

For 50- $\mu$ L rxns once the  $t=0$  point is removed

20 mg protein (40  $\mu$ L from the 0.5 mg/ $\mu$ L stock of p. 39 fr. 3)

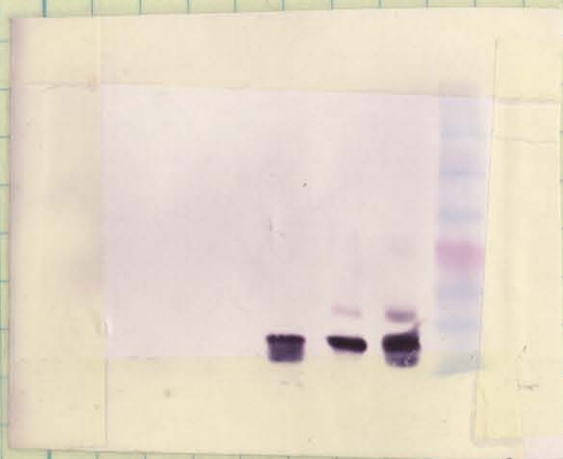
6  $\mu$ L 10x enterokinase buffer

23  $\mu$ L sterile DI water

(10  $\mu$ L of the above soln. taken for  $t=0$ )

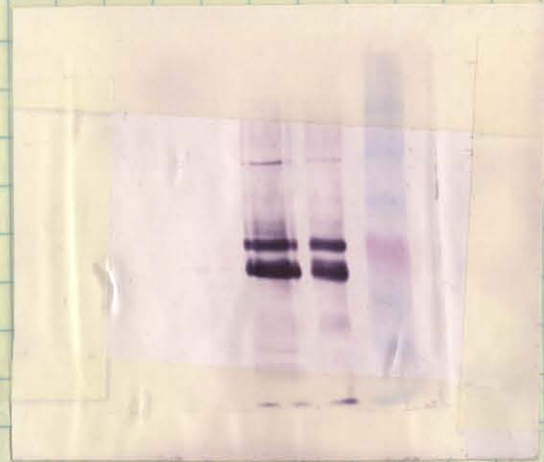
0.8 units enterokinase

The rxn tubes were flicked and incubated at rt overnight.



4 3 2 1

anti-FLAG



4 3 2 1

anti-human PAX

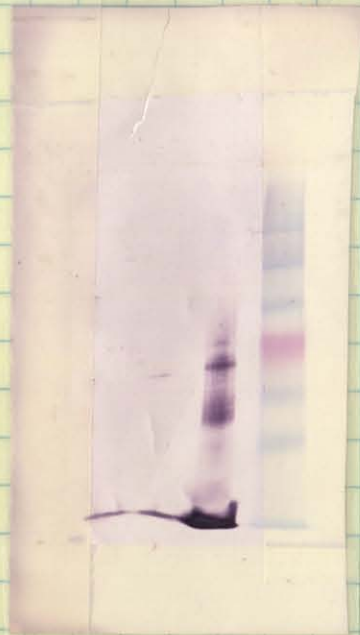
- |   |              |
|---|--------------|
| 1 | ladder       |
| 2 | $t=0$        |
| 3 | $t=5$ hours  |
| 4 | $t=20$ hours |

GST-Pax (37-558) FLAG is degrading at 4°C!

Samples that were concentrated to 1  $\mu\text{g}/\mu\text{L}$  or 0.5  $\mu\text{g}/\mu\text{L}$  in water and stored at 4°C (from the first 10-L prep) were run on gels due to the very strange results from the enterokinase cleavage test (p. 4-49).



4 3 2 1  
anti-FLAG



4 3 2 1  
anti-human PAX

In	1	ladder
	2	1 $\mu\text{g}/\mu\text{L}$ protein soln. w/ 10 $\mu\text{L}$ water
	3	0.5 $\mu\text{g}/\mu\text{L}$ protein soln. w/ 10 $\mu\text{L}$ water
	4	0.5 $\mu\text{g}/\mu\text{L}$ protein soln. w/ 10 $\mu\text{L}$ enterokinase 1 $\times$ cleavage buffer