

★ Test Cleavage with the mTEV proteinase ★ ★ ★

10 × mTEVp cleavage buffer (stored at -20°C)

500 mM Tris, pH 8.0

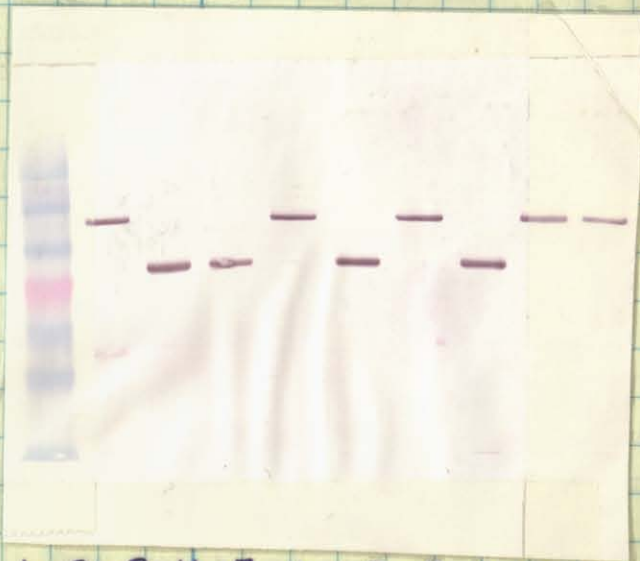
5 mM EDTA [ethylenedinitro]-tetraacetic acid disodium salt
(mw 372.24, 0.0372 g in 20 mL)

10 mM dtt (mw 154.25, 0.038 g in 20 mL)

← used here as
originally written

The following rxns were run, removing 16 μL (for 2 Western Blots) immediately before the TEVp was added, after 2 hours of incubation at the designated temp and after an additional 14 hours at room temperature.

- A: 45 μL His₆-Pa-FLAG, 5 μL buffer, 0.5 μL (4 units) mTEV, 25°C
- B: 45 μL His₆-Pax-FLAG, 5 μL buffer, 0 μL TEV, 24°C
- C: 45 μL His₆-Pax-FLAG, 5 μL buffer, 0.5 μL mTEV, 37°C
- D: 45 μL (45 μg) GST-purified only (from p. 4-89) GST-Pax-FLAG, 5 μL 1 × mTEV buffer, 0.5 μL (4 u) mTEV, 24°C
- E: 45 μL (45 μg) GST-Pax-FL, 5 μL buffer, 0.5 μL TEV, 37°C
- F: 45 μL (45 μg) GST-Pax-FL, 5 μL buffer, 1.0 μL (8 u) mTEV, 37°C
- G: 45 μL (45 μg) GST-Pax-FL, 5 μL buffer, 0 μL mTEV, 37°C



1 2 3 4 5 6 7 8 9 10
anti-FLAG 1° antibody
anti-mouse 2° antibody (alk. phos.)

- In 1 protein ladder
2 rxn D, $t=0$
3 rxn D, $t=2\text{ h}$
4 rxn D, $t=16\text{ h}$
5 rxn E, $t=0$
6 rxn E, $t=2\text{ h}$
7 rxn F, $t=0$
8 rxn F, $t=2\text{ h}$
9 rxn G, $t=0$
10 rxn G, $t=2\text{ h}$

Yea!!!



crude prot.
anti-GST →

10 9 8 7 6 5 4 3 2 1