

Tobacco Etch Virus Proteinase test cleavage

Wednesday, October 8, 2003

10 x mTEVP buffer (20 mL made):

500 mM Tris, pH 8.0

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

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

NEB usBiological mTEVP, cat. # T7950, lot # L1071301M

This mutant has reduced self-cleavage and contains a His tag.

Two 60- μ L reactions were set up both at room temp.
and (in the heating block) at 30 $^{\circ}$ C.

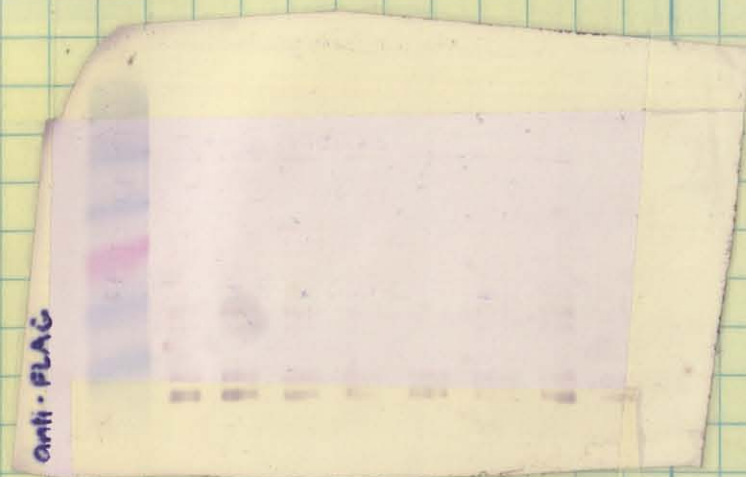
a: 20 μ L (10 μ g) 0.5 μ g/mL protein
 25 μ L sterile water
 5 μ L 10 x mTEVP buffer
 0 μ L mTEVP

} 24 $^{\circ}$ C

b: 20 μ L (10 μ g) protein soln.
 25 μ L sterile water
 5 μ L 10 x mTEV proteinase buffer
 0.4 μ L mTEVP (8 units/ μ L) 3 units

} 24 $^{\circ}$ Cc: as "a" at 30 $^{\circ}$ Cd: as "b" at 30 $^{\circ}$ C

1 2 3 4 5 6 7 8 9
 a + b (24 $^{\circ}$ C)



1 2 3 4 5 6 7 8 9
 c + d (30 $^{\circ}$ C)

In	1	10 μ L ladder	5	9 μ L b (d), t = 1 h
	2	9 μ L a (c), t = 0	6	9 μ L a (c), t = 2 h
	3	9 μ L b (d), t = 0	7	9 μ L b (d), t = 2 h
	4	9 μ L a (c), t = 1 h	8	9 μ L a (c), t = 3.75 h
			9	9 μ L b (d), t = 3.75 h