



Comparison of different protein purification procedures for TEV protease.

Plasmid pTH24TEVsh⁴² contains nt 6255–6981 from the tobacco etch virus (TEV) RNA genome⁴⁴ corresponding to aa 2038–2279 of the TEV polyprotein encoding nuclear inclusion protein A (49 kDa proteinase; NP_734212.1). The gene is flanked by attachment sites used for Gateway cloning. Nt sequences of the V5 epitope and a His₆-tag are fused to the 3' end. The plasmid was transformed into BL21(DE3) Star-Rosetta for TEV protease production. (a) Samples of TEV protease after standard ('old') and nucleic acid free ('new'; according to box 3) protein purification. 1 µg was loaded on 10% SDS-PAGE and proteins were stained with coomassie. Pictures from the two purifications were derived from the same gel, but samples in between were cut out for this illustration. (b) The same samples as in (a) were loaded on a 15% TBE-Urea polyacrylamide gel and nucleic acids were stained with SYBR gold.