

Materials & Reagents

Proteases:

1) Trypsin - 2) Trombin - 3) Subtilisyn - 4) Chymotrypsin - 5) Others

- Prepare 1mg/ml of protease in PBS buffer and make 6 dilutions:

(1mg/ml) (0.33mg/ml) (0.11mg/ml) (0.04mg/ml) (0.012mg/ml) (0.004mg/ml)

- Incubate 20µl of ~0.2mg/ml target protein with 5µl of each diluted protease, at 22°C (bath) for 30min.

- Stop incubation of all proteases by adding 6.5µl of SDS stop solution sample buffer X 5 (with 5.2mM PMSF & 5.2mM EDTA), boil at 100°C 3 to 5 min and load on gel immediately.

- Load 15µl on PAGE-SDS gel of untreated proteins and the different treated proteins.

Stop solution:

4.16µl EDTA 500mM

10.4µl PMSF 200mM

SDS-Sample buffer X 5 up to 400µl

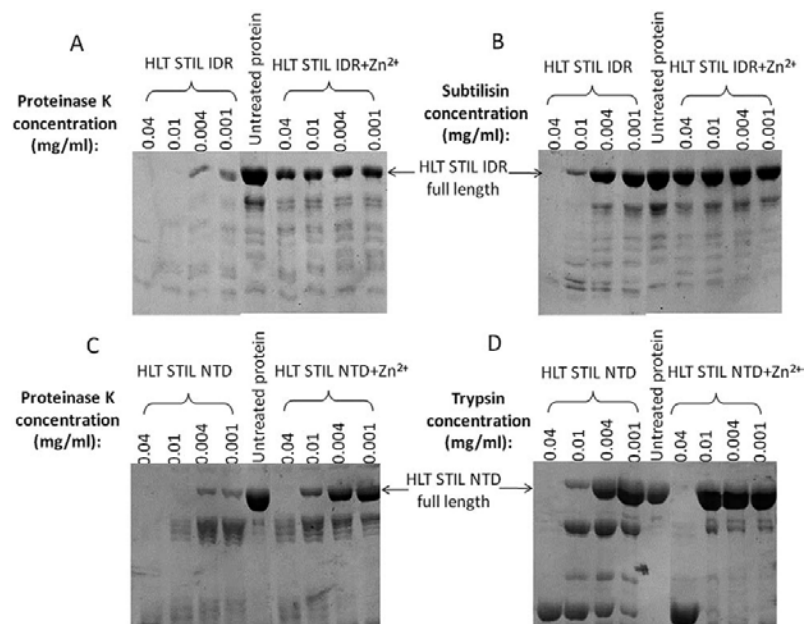


Fig.: Protease digestion of HLT STIL NTD and HLT STIL IDR: SDSPAGE gel of HLT STIL IDR with and without ZnCl₂ at several concentrations of proteinase K (A) or subtilisin (B); SDS-PAGE gel of HLT STIL NTD with and without ZnCl₂ at several concentrations of proteinase K (C) or trypsin (D).

Amartely H., David A., Shamir M., **Lebendiker M.**, Izraeli S. and Friedler A. (2016)
Differential effects of Zinc binding on structured and disordered regions in the multidomain STIL protein.
Chemical Science DOI: 10.1039/x0xx00000x

References

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