



A good starting point for optimization is to use 40 units of TEV Protease per 1 mg of target protein at 2-8 °C for 12-24 hours. Faster protein cleavage can be achieved by increasing the temperature up to 30 °C or by increasing the amount of TEV Protease to 200 units per 1 mg of target protein. Under such conditions, cleavage may be completed within 1 hour.

### On-Column Cleavage Protocol

1. Dilute the desired amount of TEV protease in a volume equal to one column volume. A concentration of 100-200 U/mL is recommended as a starting point.
2. Inject the protease solution directly onto the column.
3. Incubate the column at 4-8 °C for 8-24 hours.
4. Elute the cleaved target protein with 1-3 column volumes of elution buffer.
5. If the target protein is prone to precipitation at higher concentrations, on-column cleavage can be performed by continually circulating the protease solution in a larger volume through the column in a closed circle, until all target protein is removed from the column.

Depending on the results, the concentration of TEV Protease can be increased or decreased in subsequent experiments.

## References

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