A

Transformation into BL21 bacterial competent cells

Pre-culture overnight; inoculate large-scale culture

Grow to OD ~0.8 at 37°C; induce with IPTG at 16°C overnight

Sonication to lyse cells; benzonase treatment at 4°C

Protein was kept in high-salt buffers (500 mM NaCl or KCl) and reducing conditions throughout the purification

Two clear spins; binding incubation with Ni-NTA resin at 4°C

Resin washes; longer on-bead RNase A and benzonase wash at 4°C

Resin washes; elution; TEV protease cleavage of 6x-His tag overnight at 4°C

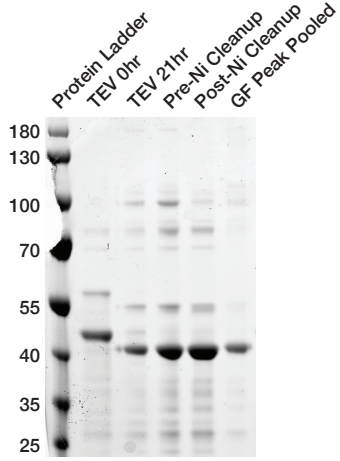
Buffer exchange into low-imidazole buffer and run back on Ni-NTA resin, keeping the supernatant

Size-exclusion chromatography (Akta); evaluate fractions, pool, and concentrate

Amine-labeling using ester dyes; dialysis at 4°C

Aliquot, flash freeze, and store in non-glycerol buffer at -80°C

B



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