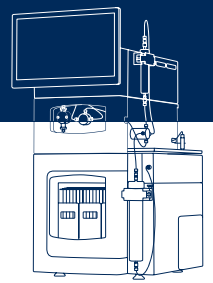
Purification of 134 Amino Acid Zinc Finger Protein using PP-15C18-F0080

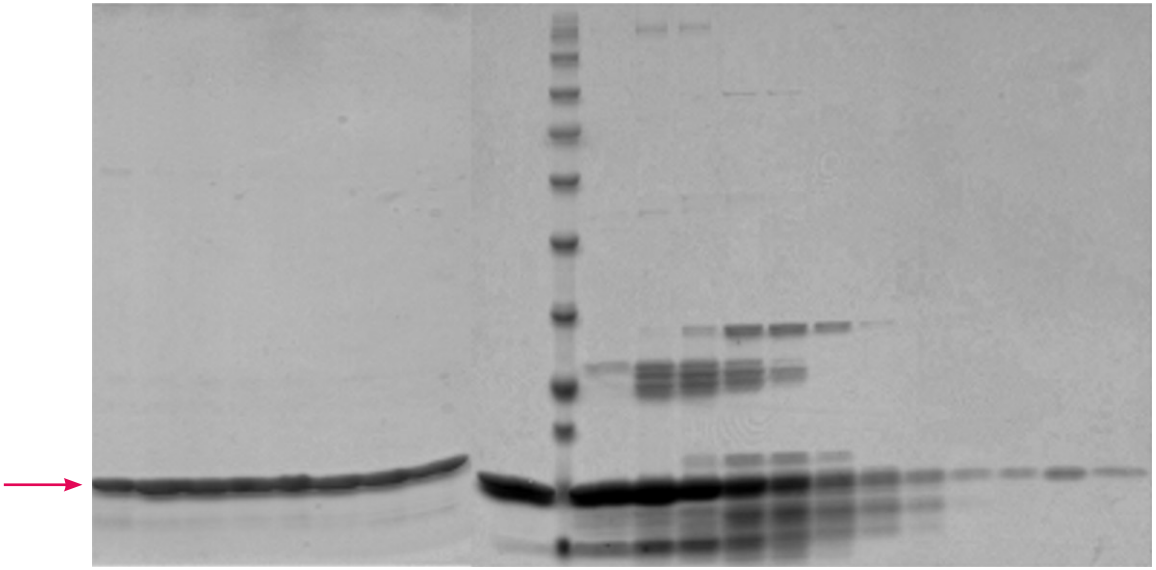
Application Note #056



1. Example data

Protein was lysed under denaturing conditions and initially purified by ion exchange chromatography.
Eluted fractions containing the protein were identified by SDS-PAGE

Ion Exchange Fractions



SDS-PAGE analysis of eluted ion exchange fractions to be further purified by RP-HPLC

FPLC fractions were combined and diluted 3-fold with solvent A (H_2O + 0.1% TFA) and filtered.
The protein solution was loaded onto the puriFlash® column.

Protein Specifications:
134 amino acid zinc finger protein isolated from *E. coli*
MW: 16.4 kDa
PI: 9.45
GRAVY hydrophobicity index: -0.7

2. Flash conditions

Device: puriFlash® 430 (or now puriFlash® 5.020)
Solvents: A: Water + 0.1%TFA
B: ACN + 0.1% TFA
(D: protein solution or H_2O for cleaning and storage)
Column: PP-15C18-F0080
Flow rate: 30mL/min
Injection mode: Liquid pump injection

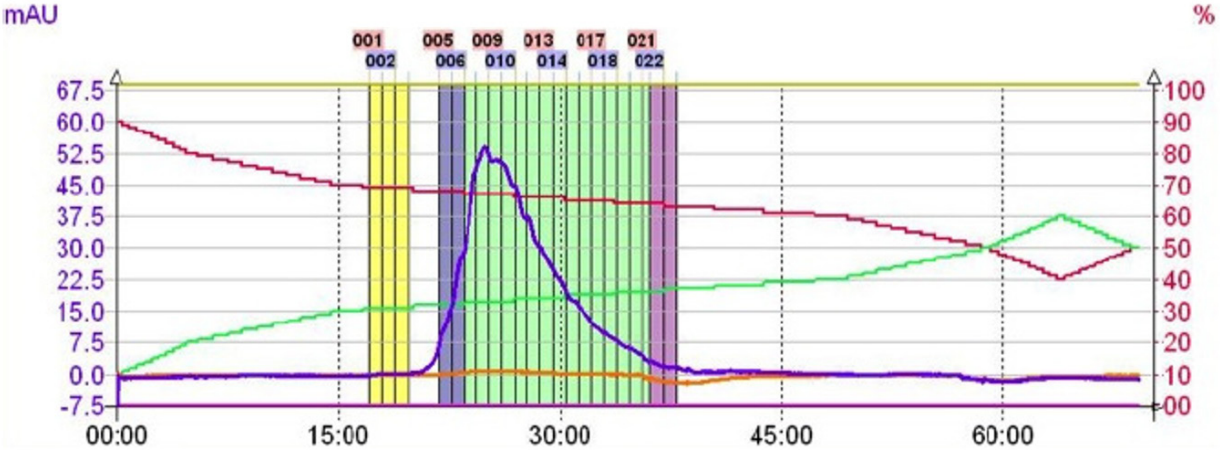
Sample Load Method					
	Flow	A	B	C	D
Initial	30mL/min	0	0	0	100
02:00:00	30mL/min	0	0	0	100

The column was then washed until the absorbance returned to baseline utilizing the following method:

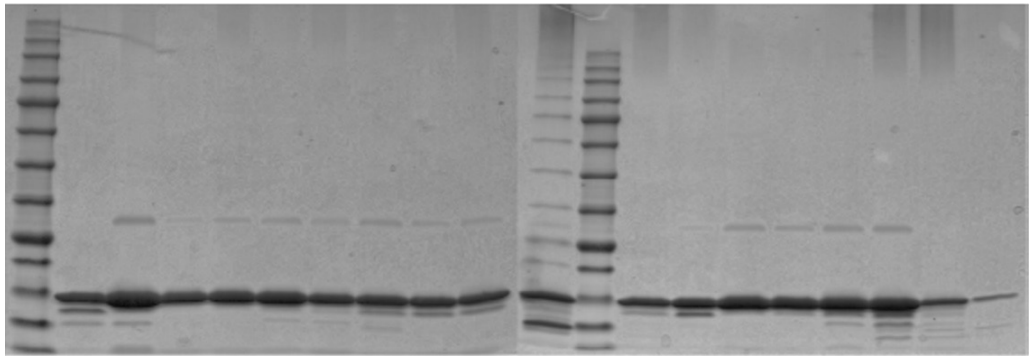
Column Wash Method			
	Flow	A	B
Initial	30mL/min	90	10
01:30:00	30mL/min	90	10

Detection: UV 220nm
Pressure: 5bar
Elution conditions:

t (min)	A (%)	B (%)
00:00:00	90	10
00:00:05	90	10
00:05:00	80	20
00:15:00	70	30
00:49:00	60	40
00:59:00	50	50
01:04:00	40	60
01:09:00	50	50
01:09:10	50	50



3. Purity confirmation with SDS-PAGE



To achieve this purification:

You will need

- puriFlash® 5.020
  Discover it Add to card
- puriFlash® column PP-15C18-F0080
  Discover it Add to card

We highly recommend

- Magic box Flash
  AXF7I0 Add to card
- 21x150mm Rack
  AYHE60 Add to card
- Tubes 21x150mm
  FL1120 Add to card