

## AKTA Prime Purification of GFP

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Clark Lab

Line "8" in lysis buffer

Line A in wash buffer

Line B in elution buffer

Method prepared for volume of column = 30 ml

Flow rate 2 ml/min

Prime all lines before starting method; empty waste bottle

Fill fraction collector with 55 tubes, rinse line to fraction collector and check drops fall in tube

Be sure Akta is communicating well with computer!

Load protein onto column manually at 2 ml/min, valve#8

Collect eluate from loading into clean tubes to check later.

Rinse protein tube with lysis buffer and load the rinse as well.

Press End to quit manual mode. Rename file to reflect the loading of sample.

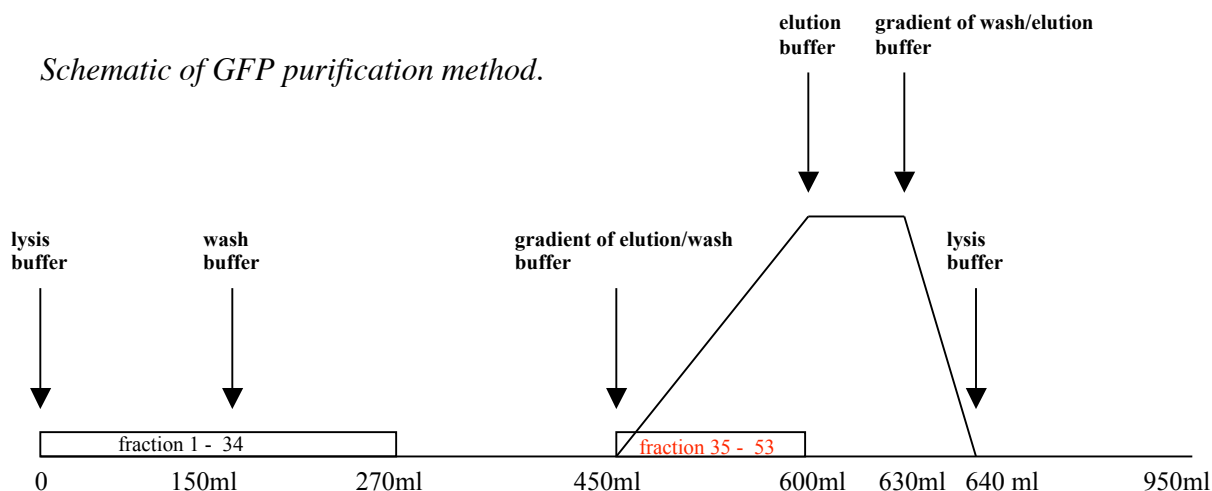
Then restart with method number 4:

- 1) 0 – 150 ml, lysis buffer and collect fractions V= 8 ml
- 2) 150 – 270 ml wash buffer and collect fractions V= 8 ml
- 3) 270 – 450 ml wash buffer, do not collect fractions
- 4) 450 – 600 ml gradient of elution buffer (150 ml, 0-100% of elution buffer), collect fractions (8 ml) with GFP
- 5) 600 – 630 ml 100% of elution buffer (to removing everything), do not collect fractions
- 6) 630 – 640 ml decrease concentration of elution buffer to 0%
- 7) 640 – 950 ml, or less, you can stop when you will see low, level absorbance

Method is now ready to load second sample of same prep; usually do 3-4 runs for one (4 L) prep.

For 1 run you need a little less than 500 ml of lysis buffer, 400 ml of wash buffer and 150 ml of elution buffer; total time for 1 run ~8 h (or less - see point 7)

*Schematic of GFP purification method.*



Clean up column for storage:

After a set of runs (one prep of up to 4 L volume), wash column with 5 volumes of H<sub>2</sub>O, setting A&B lines at 50%; also run some H<sub>2</sub>O thru the cannula on valve #8.

- then wash with 2V 0.5M NaOH, until conductivity is a flat line, use cannula on valve#8

- then through all lines: 5V H<sub>2</sub>O, then store in 30% [run thru all lines] at room temp.

Strip and regenerate column if color changes from normal blue-green.