FLAG Purification

2/98 Toshi

1. Solutions:

- Buffer H [25 mM Hepes-KOH pH7.6, 0.1 mM EDTA, 0.5 mM EGTA, 2 mM MgCl2, 20 % glycerol, 0.02 % NP40] plus KCl. Added DTT to 1 mM, sodium metabisulfite to 0.5 mM, protease inhibitor mix to final 1 X.
- Beads: FLAG **M2** beads from Kodak IBI.
- FLAG peptide (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys): 10 mg / ml stock in BufferH-0.1M KCl
- [Modification 8/00] The triple FLAG peptide works better for elution.
  Triple FLAG peptide:
  Met-Asp-Tyr-Lys-Asp-His-Asp-Gly-Asp-Tyr-Lys-Asp-His-Asp-Ile-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys. 5 mg / ml stock.

2. Procedure: all manipulations should be done in cold.

- a. Take up FLAG beads in a siliconized micro tubes. Add buffer H-0.3 to fill up the tube, turn the tube to suspend resin, and spin in Eppendorf microfuge (setting 3) for 1 min [washing]. Repeat washing 2 more times to equilibrate the beads in buffer H-0.3.
- b. Put beads in a tube containing sample solution. Do not forget to set aside a small volume of starting material for Western and staining.
- c. Rotate tube @ 4 °C for 3 hr gently (avoid forming bubbles).
- d. Spin-down beads (1,000 rpm 2~3 min. in swing bucket rotor @ 4 °C).
- e. Save sup as 'flow through'' fraction.
- f. Add ~10 ml of buffer H-0.3 to beads, rotate the tube @ 4 °C for 5 min. and centrifuge as in d.
- g. Repeat washing one more time, and transfer beads into a siliconized microfuge tube.
- h. Add buffer H-0.3 to fill up the tube, rotate it @ 4 °C for 5 min. and centrifuge in refrigerated microcentrifuge at 2,000-3,000 rpm 1 min. Remove as much buffer as possible [washing]. Do washing for total 6 times.
- i. Wash beads in buffer H-0.1M (0.2 M for ISW1 complex) three times. In this case, just invert tube a few times to suspend beads, and spin down.
- j. Add 1/5 of bed volume of 10 mg/ml FLAG peptide, mix gently, and add 4/5 of bed volume of buffer H-0.1. For triple FLAG peptide, 1mg / ml is usually enough.
- k. Rotate @ 4 °C for 30 min. Spin down in a refrigerated microcentrifuge, and save sup as an eluate.
- l. Repeat elution three more times. Last two times can be shorter than 30 min (10-15 min). Sometimes, elution at room temp works better.
- m. Analyze fractions by western and silver staining. For western, do not forget to run the starting material and flow through side-by-side to determine how much of the protein was immuno-depleted.
- n. Regenerate FLAG beads as recommended by Kodak. Store resin @ 4°C.