FLAG M2 antibody X-linking

MATERIALS

Dynabeads Protein G (Invitrogen)

anti-FLAG M2 antibodies (Sigma)

0.1M sodium phosphate pH 7.0

0.1M sodium phosphate pH 7.0, 0.01% Tween-20

0.2M triethanolamine pH 8.2 (Sigma)

20mM Dimethyl pimelimidate (0.0052g/ml) (Sigma) in 0.2M triethanolamine, pH 8.2:
must be made fresh just prior to use

50mM Tris-HCl pH 7.5

PBST: Phosphate buffered saline with 0.01% Tween-20

Magnetic particle concentrator (MPC)

PROCEDURE

1) Typically, the antibody-conjugated beads are prepared immediately before use. For each liter of yeast cells from which extract was prepared, 25 μl of Dynabeads Protein G beads slurry and 11.5 μg of anti-FLAG M2 antibodies are used. Concentrate magnetic beads on a magnetic particle concentrator (MPC), then suspend and concentrate beads twice in 0.5 ml of 0.1M sodium phosphate pH 7.0.

2) Mix antibody and magnetic beads in ~ 20 μl 0.1M sodium phosphate pH7.0 and gently shake them at room temperature for 30 minutes.

3) Suspend and concentrate beads twice in 0.5 ml of 0.1M Sodium Phosphate pH 7.0, 0.01% Tween-20.

4) Suspend and concentrate the beads twice in 0.5~1ml 0.2M triethanolamine pH 8.2.

5) Suspend the beads in 0.5~1ml of 20mM Dimethyl pimelimidate, 0.2M triethanolamine pH 8.2, and incubate them for 30 minutes at room temperature with constant rotational mixing.

6) Concentrate and suspend the beads in 0.5~1ml 50mM Tris-HCl pH 7.5 and incubate for 15 minutes at room temperature with constant rotational mixing.

7) Wash the beads three times in 0.5~1ml PBST. The beads are ready for use in purification.

Note:

a) The volume of washing solution can be 0.5~1ml, depending on the amount of beads used.

b) If antibodies other than FLAG is to be used, the optimum antibody/beads ratio should be determined empirically.