Protocol 7: **Preparation of streptavidin-coated beads**

COOH-activated glass beads were purchased from Bangs Laboratories. To coat these beads with streptavidin

7.1. Weigh 5 mg of 0.52 μm beads (catalog # SC02N) and mix with 0.5 ml of MES buffer (25 mM MES ph 5, 0.05% Tween20. Filtered through 0.2 μm pores and stored frozen in aliquots). Disperse beads until they are single by repeated rounds of pipetting through a narrow plastic tip, vortexing (5 sec at a time) and sonication (in ice cold bath, 5 min at a time).
7.2. Spin in microcentrifuge 4-6 min at 6,000 rpm and resuspend in 100 μl MES buffer
7.3. Pre-weigh SulfoNHS (Fluka, catalog #56485) and EDC (Pierce, catalog # 22980) to dissolve each in 100 μl of MES buffer (final concentration 50 mg/ml), and immediately add these solutions to beads, followed by vortexing. Incubate 30 min at room temperature on a rocker, vortexing every 5 min.
7.4. Spin and wash 3 times in 1ml of MES buffer.
7.5. Resuspend in 200 μl MES buffer with 50 μg Streptavidin. Incubate with mixing at 4^{o}C overnight.
7.6. Spin, resuspend in 40 mM glycine and incubate 20 min at room temperature to quench the reaction.
7.7. Wash 3 times and resuspend in 0.5 ml MES buffer. Beads can be stored at 4^{o}C with mixing for at least 2 months without losing their activity, as judged by staining with FITC-biotin. The resulting density of streptavidin coating is about ten-fold less than of the commercial streptavidin-coated plastic beads (Bangs Laboratories, catalog # CP01N), but it is significantly more dense and stable than the coating on the glass beads purchased from the same source.

These or commercial polystyrene streptavidin-coated beads can then be incubated with biotinylated antibodies that recognize polyhistidine tag. Wash to remove unbound antibodies and incubate with a tagged protein in a suitable buffer (e.g. Pipes or PBS). Incubate 6His proteins with these beads for 1 hour at 4^{o}C. A common problem with this procedure is that most proteins cause beads clumping. The clumping can be partially prevented by using more diluted bead and protein suspensions, adding BSA, casein or low level of detergents, and brief vortexing and sonication. Prolonged vortexing and sonication should be avoided to prevent damaging the bead-associated protein layers.