Protein-Gel Conjugation Protocol

Material:

Gel: CarboxylLink™ Coupling Gel, PIERCE#20266

Coupling buffer: PBS 0.01M

Protein: Quantified Target protein kept in water or PBS 0.01M

1. Gel Preparation
   (1) Vortex resin.
   (2) Immediately take 400ul of resin into a new Eppendorf before it precipitates.
   (3) Add 1cc of coupling buffer into the Eppendorf, and vortex for 1 min.
   (4) Centrifuge at 13000rpm for 3mins.
   (5) Discard the supernatant
   (6) Repeat step 3~5 for 20 times.
2. Protein solution preparation
   (1) Take EDC from the -20℃ fridge, and weigh 4mg on the micro-scale.
   (2) Take protein (x ul) with 100ug of protein contained in it.
   (3) Add EDC with coupling buffer(400-x ul)
   (4) Mix and vortex the EDC solution and protein together.
3. Protein-Gel incubation
   Add the protein solution into the resin gel, and react on the 3D rotary mixer overnight.(at least 12 hrs)
4. Centrifuge at 13,000rpm for 3 mins, collect the supernatant and test OD ratio via Nanodrop to check the protein incubation status.(No protein in the supernatant indicates that most protein has successfully combined to the gel.)