Coating of Antibodies or Recombinant proteins to 96-well plate surface

Procedural notes

The ability to immobilize antibodies and recombinant proteins can be useful for many research applications. In a high pH, low ionic strength solution, most proteins will coat to polystyrene plastic via ionic bonding.

Plan on working quickly once protein has been diluted into coating buffer. Depending on the protein, this dilute solution may not be stable for very long. For example: prepare a solution of 20 ml at a time to coat 2 96-well plates using a multi channel pipettor set to dispense 100ul per well. Then prepare as many other similar solutions as necessary for further coatings. Mouse IgG Antibody coated plates should be stable for at least a week in most media.

For longer term in vitro work, use of aseptic technique and sterile-filtered buffer may be appropriate.

Some recombinant proteins may lose their ability to bind to their ligand or receptor after being coated. An option to consider is capturing the protein using an alternative method such as a Streptaviden coated plate for Biotinylated products; Goat anti-Mouse coated plate for muIg containing fusion proteins; anti-muCD8 (cat # 260-020) coated plate for muCD8 containing fusion proteins.
In our experience, Mouse IgG isotype antibodies are fully functional when coated to plate surface, and should retain activity for at least a week.

In some instances antibody may be retained on cell surface after incubation.

Coating buffer
100mM Sodium Bicarbonate pH 9.2 Sterile filter (optional)

Blocking buffer
For in vitro culture work, 5%(or greater) FBS-containing culture media is appropriate to block non specific binding.
For other applications, it may be necessary to include other proteins such as 1% BSA and/or 5% non fat dry milk in PBS + 0.05% Sodium azide to adequately block wells.

Coating Procedure
Prepare dilute protein solution at final concentration of 0.5 to 10 ug/ml in coating buffer. The optimal concentration may vary from protein to protein. Often 2 ug/ml is a good starting point for Mouse IgG antibodies.
Immediately add 100 ul/well for 96-well plate wells. Incubate 2 hours RT. A longer incubation at 4^{o}C may be appropriate for some recombinant proteins.
Aspirate coating solution from wells. Add 300 ul/well of blocking reagent. Incubate 2 hours RT, or overnight at 4^{o}C.
Rinse wells one additional time with 300 ul of media (Cell culture) or wash buffer (for EIA) prior to use.