INSTRUCTIONS

Pierce® Protein A, Protein G and Protein A/G Coated 96-Well Plates

0657.4

Number	Description
15130	**Pierce Protein A Coated Plates (clear, 96-well),** 5 each
15132	**Pierce Protein A Coated Plates (clear, 8-well strips),** 5 each
15154	**Pierce Protein A Coated Plates (white, 96-well),** 5 each
15155	**Pierce Protein A Coated Plates (black, 96-well),** 5 each Binding Capacity: ~4pmol rabbit IgG/well
15131	**Pierce Protein G Coated Plates (clear, 96-well),** 5 each
15133	**Pierce Protein G Coated Plates (clear, 8-well strips),** 5 each
15156	**Pierce Protein G Coated Plates (white, 96-well),** 5 each
15157	**Pierce Protein G Coated Plates (black, 96-well),** 5 each Binding Capacity: ~2pmol rabbit IgG/well
15138	**Pierce Protein A/G Coated Plates (clear, 8-well strips),** 5 each Binding Capacity: ~5pmol rabbit IgG/well

Note: Plates are activated to 100µL and supplied pre-blocked with Thermo Scientific SuperBlock Blocking Buffer. Use these plates for single-antibody assays only. In multiple antibody assays, such as sandwich ELISAs, the first antibody cannot block all binding sites and subsequent antibodies will bind to the plate, resulting in false positives.

Storage: Upon receipt store plates at 4°C in unopened pouches. Place opened, unused plates in a resealable bag and store desiccated at 4°C. Plates are shipped at ambient temperature.

Introduction

Microplates coated with Proteins A, G and A/G specifically bind the Fc region of immunoglobulins resulting in antibody orientation such that the antigen binding site is directed away from the microplate and available for binding. These coated plates can increase antibody-binding capacity compared to coating antibodies directly to the plates.

These Thermo Scientific Pierce Coated Plates are useful for detecting antibodies using labeled antigens. The pre-coated plates can increase assay sensitivity and are ideal when a low amount of antibody is available. Antibodies bind to Protein A, Protein G or Protein A/G rapidly and dissociate with strong chaotropes and extreme pH. The coated plates are available in clear for colorimetric assays, white for chemiluminescent assays, and black for fluorescent assays.

The antibody being used determines whether to use Protein A, Protein G or Protein A/G coated plates. Protein G binds all subclasses of mouse, rabbit and goat IgG as well as most other commonly used species. Protein A binds strongly to rabbit antibodies and binds pig and guinea pig IgG better than Protein G; Protein A does not bind mouse IgG_1 well, and has weak binding of most rat IgG subclasses. Protein A/G is a secreted gene fusion product from a nonpathogenic form of *Bacillus* and contains four Fc-binding domains from Protein A and two from Protein G making it a more versatile tool. Please see the Pierce catalog website or for a list of binding characteristics of the different binding proteins.

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Example Procedure for ELISA-based Applications

The following procedure is an example ELISA protocol. Specific applications and systems will require optimization. Do not use these plates for multiple antibody assays, such as sandwich ELISAs, because the first antibody will not block all binding sites and subsequent antibodies can still bind to the plate, resulting in false positives.

Note: For binding information concerning the various proteins, please see Tech Tip #34: Binding characteristics of Protein A, G, A/G and L from our website.

Additional Materials Required

- Capture antibody specific for the antigen of interest
- Wash Buffer: TBS (Product No. 28376) or PBS (Product No. 28374) with added 0.05% Tween®-20 Detergent
- Dilution Buffer: SuperBlock® Blocking Buffer (Product No. 37535) containing 0.05% Tween-20 Detergent (Product No. 28320) or other proteinaceous blocking solution
- Antigen labeled with HRP, alkaline phosphatase or biotin
- Enzyme substrate (see our catalog or website for a complete product listing)

Procedure

1. Rinse each well three times with 200µL of Wash Buffer.
2. Using the Dilution Buffer dilute antibody to ~1µg/mL and add 100µL to each well.
3. Incubate plate 30-60 minutes at room temperature. For best results, for all microplate incubations use a plate mixer that creates a vortex in each well.
4. Rinse each well three times with 200µL of Wash Buffer.
5. Add the labeled antigen to each well (~0.1µg/mL). Sample may be diluted in Dilution Buffer. Incubate at 37°C for 1 hour.
6. Rinse each well three times with 200µL of Wash Buffer.
7. If using a biotinylated antigen, add enzyme-labeled streptavidin or other biotin-binding protein and incubate at 37°C for 1 hour. Rinse each well three times with 200µL of Wash Buffer.
8. Detect the signal according to the instructions for the specific detection system being used.

Related Thermo Scientific Products

34028	**1-Step Ultra TMB-ELISA,** 250mL
34022	**1-Step Turbo TMB,** 250mL
37621	**1-Step PNPP,** 100mL
15075	**Reagent Reservoirs,** 200/pkg
15036	**Sealing Tape for 96-Well Plates,** 100/pkg

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