INSTRUCTIONS

Pierce™ Protein L Coated Plates

15190 0779.2

Number	Description
15190	**Pierce Protein L Coated Plates,** 5 plates

Note: Plates are activated to 100µL and supplied pre-blocked with 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

The Thermo Scientific Pierce Protein L 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 L rapidly and dissociate with strong chaotropes and extreme pH.

Protein L is an immunoglobulin-binding protein that was isolated from the bacteria *Peptostreptococcus magnus* and is now produced recombinantly. Protein L binds to immunoglobulin kappa light chains without interfering with the antigen-binding site and binds a wider range of Ig classes and subclasses than other antibody-binding proteins such as Protein A or Protein G. Protein L binds to all classes of Ig (i.e., IgG, IgM, IgA, IgE and IgD). Protein L also binds single chain variable fragments (scFv) and Fab fragments.

Important Product Information

- Protein L **only** binds to immunoglobulins containing light chains of type kappa I, III, and IV in human and kappa I in mouse. Protein L also may be specific for certain kappa subgroups in other species. Protein L binds scFv without interfering with antigen binding.
- Protein L binds weakly to rabbit immunoglobulins and does not bind immunoglobulins from bovine, goat or sheep; nor does it bind to lambda light chains.

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 the website for Tech Tip #34: Binding characteristics for Immunoglobulin Binding Proteins (Protein A, G, A/G and L).

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: Thermo Scientific 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

- Appropriate substrate for the enzyme (see Related Thermo Scientific Products Section or 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 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.
15130	**Pierce Protein A Coated Plates (clear, 96-well),** 5 each
15131	**Pierce Protein G Coated Plates (clear, 96-well),** 5 each
15138	**Pierce Protein A/G Coated Plates (clear, 8-well strips),** 5 each

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Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.