INSTRUCTIONS

Pierce® Antibody Coated 96-Well Plates

0687.3

Number	Description
15134	**Pierce Goat Anti-Mouse IgG Coated Plates (clear, 96-well),** 5 plates
15234	**Pierce Goat Anti-Mouse IgG Coated Plates (white, 96-well),** 5 plates
15334	**Pierce Goat Anti-Mouse IgG Coated Plates (black, 96-well),** 5 plates
	Binding Capacity: ~7pmol IgG/well
15135	**Pierce Goat Anti-Rabbit IgG Coated Plates (clear, 96-well),** 5 plates
15136	**Pierce Goat Anti-Rabbit IgG Coated Plates (white, 96-well),** 5 plates
15137	**Pierce Goat Anti-Rabbit IgG Coated Plates (black, 96-well),** 5 plates
	Binding Capacity: ~12pmol IgG/well

Note: Plates are activated to 100µL and supplied pre-blocked with SuperBlock® Blocking Buffer.

Storage: Upon receipt store plates at 4°C in unopened pouches. Once opened, place unused plates in a resealable bag and store desiccated at 4°C.

Introduction

The Thermo Scientific Pierce Antibody Coated Plates are ideal for binding assays when available antibodies are in low quantities or denature upon direct adsorption to polystyrene plates.[1-4] Because these plates are IgG-specific, purified antibodies are not required. Pierce Antibody Coated Plates may be used for direct, indirect, competitive or sandwich assays and are available in clear for colorimetric assays, white for chemiluminescent assays, and black for fluorescent assays.

Note: The given protocols below are general ELISA procedures. Specific applications require optimization.

General Procedure for Direct ELISA

A. Materials Required

- Antigen-specific antibody
- Biotin-labeled antigen
- Dilution Buffer: Thermo Scientific StartingBlock T20 (PBS) Blocking Buffer (Product No. 37539), contains blocking buffer with 0.05% Tween®-20 Detergent
- Wash Buffer: Tris-buffered Saline (TBS, Product No. 28376) or Phosphate Buffered Saline (PBS, Product No. 28374) containing 0.05% Tween-20 Detergent (Product No. 28320)
- Detecting Enzyme: Streptavidin or Thermo Scientific NeutrAvidin Biotin-Binding Protein labeled with HRP (Product No. 21124 or 31001) or other enzyme
- Enzyme Substrate (e.g., for HRP use Thermo Scientific TMB Substrate Kit, Product No. 34021)

B. Method

Note: Plates are supplied pre-blocked with Thermo Scientific SuperBlock Blocking Buffer.

1. Rinse each well three times with 200µL of Wash Buffer.
2. Dilute antibody to 0.1-1µg/mL with Dilution Buffer and add 100µL to each well.

3. Incubate plate for 30-60 minutes at room temperature. For all incubation steps use a plate mixer at a speed that creates a vortex in each well.
4. Rinse each well three times with 200µL of Wash Buffer. Add 100µL of the biotinylated antigen to each well (approximately 0.1µg/mL). Incubate plate at 37°C for 1 hour.
5. Rinse each well three times with 200µL of Wash Buffer. Add 100µL of the Detecting Enzyme at 0.1-1µg/mL and incubate at 37°C for 1 hour.
6. Rinse each well three times with 200µL of Wash Buffer.
7. Develop and evaluate plate according to the instructions for the specific enzyme substrate.

General Procedure for Indirect ELISA

A. Materials Required

- Matched antibody pair: antigen-specific capture antibody and antigen-specific enzyme-labeled antibody
- Antigen
- Dilution Buffer: StartingBlock T20 (PBS) Blocking Buffer (Product No. 37539), contains blocking buffer with 0.05% Tween-20 Detergent
- Wash Buffer: Tris Buffered Saline (TBS, Product No. 28376) or Phosphate Buffered Saline (PBS, Product No. 28374) containing 0.05% Tween-20 Detergent (Product No. 28320)
- Enzyme Substrate (e.g., for HRP use TMB Substrate Kit, Product No. 34021)

B. Method

Note: Plates are supplied pre-blocked with SuperBlock Blocking Buffer.

1. Rinse each well three times with 200µL of Wash Buffer.
2. Dilute the capture antibody to 0.1-1µg/mL with Dilution Buffer and add 100µL to each well.
3. Incubate plate for 30-60 minutes at room temperature. For all incubation steps use a plate mixer at a speed that creates a vortex in each well.
4. Rinse each well three times with 200µL of Wash Buffer. Add 100µL (~0.1µg/mL) of antigen to each well. Incubate plate at 37°C for 1 hour.
5. Rinse each well three times with 200µL of Wash Buffer. Add 100µL (0.1-1µg/mL) of the enzyme-labeled detection antibody and incubate at 37°C for 1 hour.
6. Rinse each well three times with 200µL of Wash Buffer.
7. Develop and evaluate plate according to the instructions for the specific enzyme substrate.

Related Thermo Scientific Products

37070	**SuperSignal® ELISA Pico Chemiluminescent Substrate,** 100mL, peroxidase substrate
15169	**QuantaBlu™ Fluorogenic Peroxidase Substrate Kit**
34028	**1-Step™ Ultra TMB-ELISA,** 250mL, colorimetric peroxidase substrate
37621	**1-Step PNPP,** 100mL, colorimetric phosphatase substrate
15075	**Reagent Reservoirs,** 200/pkg
15082	**Microtube Racked System,** 960 tubes
15036	**Sealing Tape for 96-Well Plates,** 100/pkg
21425	**EZ-Link® Sulfo-NHS-Biotinylation Kit**
21335	**EZ-Link Sulfo-NHS-LC-Biotin,** 100mg, biotinylation reagent with 22.4Å spacer arm
21126	**Streptavidin, Horseradish Peroxidase Conjugated,** 1mg

Cited References

1. Cantarero, L.A., *et al*. (1980). The adsorptive characteristics of protein for polystyrene and their significance in solid-phase immunoassays. *Anal Biochem* **105:**375-82.
2. Dierks, S.E., *et al*. (1986). Altered recognition of surface-absorbed compared to antigen-bound antibodies in the ELISA. *Mol Immunol* **23:**403.
3. Shields, M.J., *et al*. (1991). An appraisal of polystyrene- (ELISA) and nitrocellulose-based (ELIFA) enzyme immunoassay systems using monoclonal antibodies reactive toward antigenically distinct forms of human C-reactive protein. *J Immunol Meth* **141:**253-61.
4. Schwab, C. and Bosshard, H.R. (1992). Caveats for the use of surface-absorbed protein antigen to test the specificity of antibodies. *J Immunol Meth* **147:**125-34.