Xpress Capture Protein A Coated Microplates

An affinity-capture surface for oriented antibody immobilization and labeled-antigen detection

Catalog Numbers:

EX6000-C96 (Clear 96 well plate)
EX6000-B96 (Black 96 well plate)
EX6000-W96 (White 96 well plate)
EX6000-C384 (Clear 384 well plate)
EX6000-B384 (Black 384 well plate)
EX6000-W384 (White 384 well plate)

Product Description

Express Capture Protein A Coated Microplates provide a solid-phase surface for capturing immunoglobulins through affinity interactions between immobilized Protein A and the Fc region of compatible antibodies. Fc-directed capture helps position the antigen-binding regions away from the plate surface so they remain available to bind target molecules in solution.
Protein A binds immunoglobulins in a species-, class-, and subclass-dependent manner. It is commonly used with rabbit IgG and may also provide useful binding with antibodies from several other mammalian species. Protein A generally binds mouse IgG1 and most rat IgG subclasses weakly; compatibility should therefore be confirmed for the antibody used in the assay.
The plates are intended for research immunoassays in which an immobilized antibody captures a labeled or otherwise detectable antigen. Detection may be performed with enzyme-labeled, biotinylated, fluorescent, chemiluminescent, or comparable reagents. Conditions should be optimized for each antibody, antigen, label, and sample matrix.

Components

Five Protein A coated 96-well microplate supplied in a sealed foil pouch with desiccant. Plates may be supplied as a solid plate or as removable strips, depending on the catalog configuration. Refer to the product label for the supplied format and expiration date.

Storage and Handling

Store the unopened plate at 2-8 C. Do not freeze. Allow the sealed pouch to reach room temperature before opening to reduce condensation on the wells. Return unused strips promptly to the pouch with desiccant, reseal securely, and store at 2-8 C. Protect the plate from prolonged exposure to moisture.

Materials and Equipment Required but Not Provided

- Compatible primary antibody
- Labeled or biotinylated antigen
- Phosphate-buffered saline or other validated assay buffer
- Blocking or antibody diluent prepared fresh as required
- Wash buffer such as PBS containing 0.05% Tween 20
- Streptavidin-enzyme conjugate when using a biotinylated antigen
- Appropriate substrate and stop solution when required
- Microplate washer or wash bottle
- Calibrated single- and multichannel pipettes
- Microplate reader appropriate for the selected detection system

Buffers:

PBS: Phosphate-buffered saline, pH approximately 7.2-7.4.

PBST: PBS containing 0.05% Tween 20 unless otherwise validated.

Antibody Diluent: PBS containing 1% bovine serum albumin is a recommended starting formulation. Alternative blockers or diluents may be evaluated when background or analyte recovery requires optimization.

Recommended Concentrations:

Antibody: Approximately 1 microgram/mL. A titration should be performed to determine the concentration that provides the best signal-to-background ratio for the antibody and application.

Labeled Antigen: Approximately 0.1 microgram/mL. The optimal concentration depends on antigen labeling, binding affinity, specific activity, and the selected detection system.

Protocol:

1. Bring the sealed plate and required reagents to room temperature before use.
2. Prepare the antibody in antibody diluent at an appropriate concentration. Approximately 1 microgram/mL is recommended as a starting concentration. Add 100 microliters of the diluted antibody to each assigned well.
3. Cover the plate and incubate for 30-60 minutes at room temperature. Gentle plate agitation may be used if validated.
4. Wash each well with 300 microliters of Wash Buffer and remove the residual liquid after the wash. Repeat twice for a total of three washes.
5. Prepare the labeled or biotinylated antigen in the selected assay diluent. Approximately 0.1 microgram/mL is recommended as a starting concentration. Add 100 microliters of the antigen solution to each well.
6. Cover the plate and incubate for 60 minutes at room temperature unless an alternative time and temperature have been validated.
7. Wash each well with 300 microliters of Wash Buffer and remove the residual liquid after each wash. Repeat for a total of three to five washes.
8. For a directly labeled antigen, proceed to Step 10. For a biotinylated antigen, add 100 microliters of the appropriately diluted streptavidin-enzyme conjugate to each well. Optimization may be required to determine the appropriate conjugate concentration for the assay. Incubate according to the conjugate manufacturer's instructions.
9. Wash each well with 300 microliters of Wash Buffer and remove the residual liquid after each wash. Repeat for a total of three to five washes.

10. Add the appropriate substrate to each well and incubate for 5-30 minutes, or until the desired signal develops. Stop the reaction when required and read the plate at the wavelength specified for the selected substrate and detection system.

Controls

Include a known reactive antibody control, a nonreactive antibody control when available, and a reagent blank that receives diluent in place of antibody. Additional controls may be required for the antigen, label, conjugate, or sample matrix. Interpret results only when the controls perform within established acceptance criteria.

Critical Steps

1. Confirm that the antibody species and IgG subclass are compatible with Protein A before beginning the assay.
2. Do not allow wells to dry between assay steps. Drying can increase background and well-to-well variability.
3. Use consistent pipetting, incubation timing, temperature, and washing across the plate.
4. Remove residual wash buffer after each wash cycle, but avoid striking the plate hard enough to dislodge removable strips.
5. High background may result from excessive antibody or antigen concentration, inadequate washing, unsuitable blocking conditions, or an overly concentrated detection reagent.
6. Low signal may result from poor Protein A compatibility, insufficient antibody or antigen concentration, loss of antigen activity, or an inappropriate detection reagent dilution.
7. Determine optimal reagent concentrations experimentally using checkerboard titration when developing a new assay.

Limitations

Protein A affinity varies by immunoglobulin species, class, and subclass. Plate binding does not by itself demonstrate that an antibody retains functional antigen-binding activity. Performance characteristics must be established for each assay, sample type, and detection system.

Troubleshooting Guide

High background: Increase the number or volume of wash steps; reduce the antibody, antigen, or detection-reagent concentration; evaluate an alternative blocker; and confirm that wells are not drying during the procedure.

Low or absent signal: Verify Protein A compatibility with the antibody species and subclass; confirm antigen integrity and label activity; increase the antibody or antigen concentration within an experimentally justified range; and verify the detection-system settings.

High well-to-well variation: Confirm calibrated pipette performance, consistent incubation times, complete liquid removal after washing, uniform reagent temperature, and absence of bubbles before reading the plate.

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