INSTRUCTIONS

Pierce® Amine-binding, Maleic Anhydride 96-Well Plates

0462.7

Number	Description
15100	**Pierce Amine-binding, Maleic Anhydride Activated Plates (clear, 8-well strips),** 5 each
15102	**Pierce Amine-binding, Maleic Anhydride Activated Plates (clear, 8-well strips),** 5 × 5 each
15110	**Pierce Amine-binding, Maleic Anhydride Activated Plates (clear, 96-well),** 5 each
15112	**Pierce Amine-binding, Maleic Anhydride Activated Plates (clear, 96-well),** 5 × 5 each
15108	**Pierce Amine-binding, Maleic Anhydride Activated Plates (white, 96-well),** 5 each

Activation Level: 200µL

Binding Capacity: ~125pmol biotin pentylamine (328Da)/well

Storage: Upon receipt store plates at room temperature. Product shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce Maleic Activated Plates allow attachment of amine-containing peptides and other molecules to microplate wells for use in binding assays. The plates are useful for immobilizing peptides and other ligands that do not coat efficiently by passive adsorption. Reaction of the maleic anhydride groups with primary amines (-NH_2) results in formation of amide bonds that are stable at neutral pH and above (Figure 1). Acidic conditions hydrolyze the bond; for example, the half-life of hydrolysis at pH 3.5 and 37°C is approximately 11 hours.[1] Therefore, coating reactions are best performed at pH 8-9, and the reacted plates are best used at pH > 7 for ELISA and other methods.

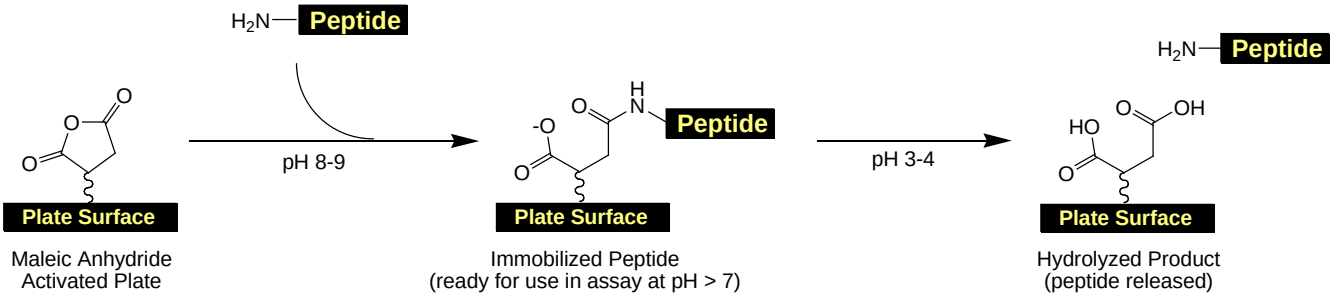

Figure 1. Immobilization reaction of Maleic Anhydride Activated Plate to an amine-containing molecule.

Procedure for Immobilizing Amine-containing Peptides

A. Additional Materials Required

- Immobilization Buffer: Phosphate-buffered saline (PBS: 0.1M sodium phosphate, 0.15M sodium chloride, pH 7.2; Product No. 28372) or other alkaline, amine-free buffer such as carbonate/bicarbonate buffer (0.2M, pH 9.4; Product No. 28382). Do not use Tris-based buffer because it contains primary amines that compete with the intended reaction.
- Amine-containing peptide or other ligand for immobilizing to the plate
- Protein Blocking Buffer: (e.g., Thermo Scientific SuperBlock Blocking Buffer, Product No. 37515 or 37535)
- Wash Buffer: PBS containing 0.05% Tween®-20 Detergent (Product No. 28320)

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B. Procedure

1. Wash plate wells three times with 200µL each of Wash Buffer.
2. Dissolve peptide at 10µg/mL in the Immobilization Buffer.

   Note: Optimize coating concentration by testing several dilutions of the ligand or peptide (e.g., 1, 2, 5, 10 and 20µg/mL).
3. Add 100µL of peptide solution to each well.
4. Incubate plate on a shaker for 1 hour at room temperature or 37°C. For maximum binding, incubate plate overnight.
5. Remove peptide solution from plate wells.
6. Add at least 200µL of Protein Blocking Buffer per well and incubate plate for 1 hour at room temperature. This step quenches remaining reactive maleic anhydride groups and also blocks remaining open sites on the plate surface.
7. Discard Protein Blocking Buffer and wash plate wells several times with Wash Buffer.
8. Proceed with ELISA or other assay method. Plates that have been blocked with SuperBlock® Blocking Buffer often can be dried and stored at 4°C for several months without affecting ELISA detection.

Related Thermo Scientific Products

Refer to a current catalog or the web site for additional information about ELISA-related products, including wash buffers and additives, blocking buffers, secondary antibodies and substrates.

Cited Reference

1. Means, G.E. and Feeney, R.E. (1971). Protein Modification. Holden Day, Inc., San Francisco, CA.

Product References

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