

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 (-NH₂) 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.

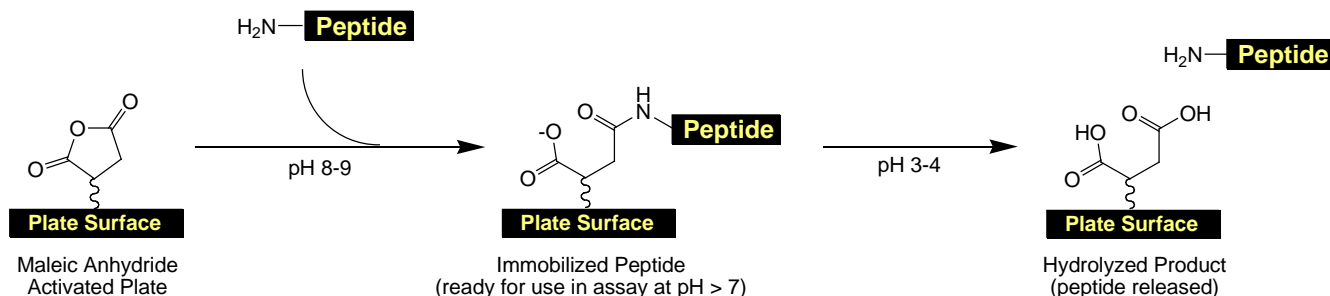


Figure 1. Immobilization reaction of Maleic Anhydride Activated Plate to an amine-containing molecule.

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)

B. Procedure

1. Wash plate wells three times with 200 μ L each of Wash Buffer.
2. Dissolve peptide at 10 μ g/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 20 μ g/mL).
3. Add 100 μ L 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 200 μ L 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

- Aufderheide, A.C., *et al.* (2004). A 9,000-year record of Chagas' disease. *Proc Natl Acad Sci USA*. **101**:2034-9.
- Brett, P.J., *et al.* (2002). Characterization of oligopeptides that cross-react with carbohydrate-specific antibodies by real time kinetics, in-solution competition enzyme-linked immunosorbent assay, and immunological analyses. *J Biol Chem* **277**:20468-76.
- Gregory, K. and Mello, C.M. (2005). Immobilization of *Escherichia coli* cells by use of the antimicrobial peptide cecropin P1. *Appl Envir Microbiol* **71**:1130-4.
- Kojima, K. *et al.* (2004). Podocyte flattening and disorder of glomerular basement membrane are associated with splitting of dystroglycan-matrix interaction. *J Am Soc Nephrol* **15**:2079-89.
- Leu, S-J., *et al.* (2003). Identification of a novel integrin $\alpha_6\beta_1$ binding site in the angiogenic inducer CCN1 (CYR61). *J Biol Chem* **278**:33801-8.
- Li, Y., *et al.* (2003). Sequences within domain II of the urokinase receptor critical for differential ligand recognition. *J Biol Chem* **278**:29925-32.
- Liao, Y-F., *et al.* (2002). The EIIIA segment of fibronectin is a ligand for integrins $\alpha_6\beta_1$ and $\alpha_4\beta_1$ providing a novel mechanism for regulating cell adhesion by alternative splicing. *J Biol Chem* **277**:14467-74.
- Miura, Y., *et al.* (2002). Analysis of the interaction of platelet collagen receptor glycoprotein VI (GPVI) with collagen. A dimeric form of GPVI, but not the monomeric form, shows affinity to fibrous collagen. *J Biol Chem* **277**:46197-204.
- Sompuram, S.R., *et al.* (2002). Synthetic peptides identified from phage-displayed combinatorial libraries as immunodiagnostic assay surrogate quality-control targets. *Clin Chem* **48**:410-20.
- von Büdingen, H-C., *et al.* (2002). Molecular characterization of antibody specificities against myelin/oligodendrocyte glycoprotein in autoimmune demyelination. *Proc Natl Acad Sci USA*. **99**:8207-12.

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