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Well-Coated™ Sulfhydryl Binding

96-well plates for binding peptide & protein free sulfhydryl groups

INTRODUCTION

Well-Coated™ Sulfhydryl Binding plates are designed to specifically bind free sulfhydryls of peptides, proteins and other molecules. The *Well-Coated*™ Sulfhydryl Binding plates are designed to overcome the inherent issues of passive adsorption for immobilizing peptides and other ligands for binding assays.

Well-Coated™ Sulfhydryl Binding plates are maleimide activated plates that react with free sulfhydryls to form stable thioether bonds at pH 6.5-7.5. pH >7.5 significantly increases the reaction of amines with the maleimide groups.

The wells are coated to a 100µl depth and are supplied pre-blocked in our proprietary *Superior*™ Blocking Buffer. The clear, white and black plates are offered for colorimetric, chemiluminescence and fluorescent detection systems, respectively.

KIT COMPONENTS

Cat. #	Components	Size
786-754	*Well-Coated*™ Sulfhydryl Binding, 96 well plate	5 plates
786-755	*Well-Coated*™ Sulfhydryl Binding, 8-well strip plate	5 plates
786-780	*Well-Coated*™ Sulfhydryl Binding, 96 well plate, Black	5 plates
786-781	*Well-Coated*™ Sulfhydryl Binding, 96 well plate, White	5 plates

STORAGE CONDITIONS

Shipped at ambient temperature. Upon arrival, store unopened at 4°C. Once opened the plates can be stored in a resealable bag (ZipLoc) with an appropriate desiccant at 4°C.

BINDING CAPACITY

***Well-Coated*™ Sulfhydryl Binding:** ~120pmol sulfhydryl peptide/well

PROTOCOL

The following protocol is a simple direct ELISA protocol and the protocol and reagents used will have to be optimized for specific applications and assays.

ITEMS NEEDED BUT NOT SUPPLIED

- Binding Buffer: We recommend our Optimizer Buffer™ III (Cat. # BKC-06) that is specifically designed for sulfhydryl coupling reactions. Alternatively 0.1M sodium phosphate, 0.15M NaCl and 10mM EDTA, pH 7.2 can be used.
- Peptide, protein or other ligand with free primary amine

  NOTE: Ellman's Reagent (Cat. # BC87) can be used to determine the amount of free sulfhydryls.

  Several reducing agents are available to reduce oxidized peptides /proteins to generate free sulfhydryls (see Related Products).

  For peptides or proteins lacking sulfhydryls, SATA (N-Succinimidyl-S-acetylthioacetate) (Cat. # BC96) or Traut's Reagent (2-Iminothiolane hydrochloride) (Cat. # BC95) can be used to add sulfhydryls via amine modification.

- Wash Buffer: femtoTBST™ (Cat. # 786-161) or femtoPBST™ (Cat. # 786-162); 10X concentrated wash buffers supplemented with Tween® 20. Or an appropriate wash buffer of choice.
- Cysteine•HCl to block unreacted maleimide sites
- Blocking Buffer: A suitable blocking buffer, we recommend our *Superior*™ Blocking Buffer (Cat. # 786-655 to 786-661) or NAP-BLOCKER™, an animal free blocking agent suitable for ELISA (Cat. # 786-190).
- Primary and labeled secondary antibodies
- Detection system for label, femtoELISA™ is a chromogenic detection system for HRP and AP (Cat. # 786-110 to 786-113)

Direct ELISA Assay

1. Wash the wells to be used three times with 200µl Wash Buffer.
2. Dilute the peptide to be bound to 1-50µg/ml in Binding Buffer. Add 100µl to each well.

   NOTE: The amount of peptide to be added needs to be optimized by using various peptide concentrations.
3. Incubate at room temperature for >120 minutes at 37°C, for optimal binding use a plate shaker and incubate overnight at 4°C.
4. Remove the peptide solution and wash each well three times with 200µl Wash Buffer.
5. Immediately prior to use, prepare a 1oµg/ml cysteine solution and add 200µl to each well. Incubate for 1 hour at room temperature.
6. Continue with the ELISA or other assay.

RELATED PRODUCTS

I. ***Reducing Agents:***
   - *a.* ***β-Mercaptoethanol (Cat. # BC98)***
   - *b.* ***DTT (Cat. # 786-227)***
   - *c.* ***TCEP (Cat. # 786-230)***

II. ***Ellman's Reagent (Cat. # BC87:*** *For the measurement of free sulfhydryl groups in protein and peptide solutions.*

For a wide range of ELISA products, including blocking buffers, wash buffers and other Well-Coated™ plates visit **www.GBiosciences.com** for more details.

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