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A Geno Technology, Inc. (USA) brand name

Well-Coated™ Sulfhydryl Binding

96-Well Plates for Binding Peptide & Protein Free Sulfhydryl Groups

(Cat. # 786-754, 786-755, 786-780, 786-781)

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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. The clear, white and black plates are offered for colorimetric, chemiluminescence and fluorescent detection systems, respectively.

KIT COMPONENTS

Cat. #	Components	Size
786-755	*Well-Coated*™ Sulfhydryl Binding, 8-well strip plate, Clear	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

IMPORTANT INFORMATION

1. Maleimides 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.
2. Some sulfhydryl-containing peptides and proteins may oxidize in solution and form disulfide bonds, which cannot react with maleimides. Disulfide bonds can be reduced to produce free sulfhydryls. The G-Biosciences Immobilized Reductant (Cat. # 786-148) enable peptide or protein reduction while recovering the sample in the absence of reducing agents.
3. Ellman's Reagent (Cat. # BC87) can be used to determine the amount of free sulfhydryls. (See Appendix)
4. 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. (See Appendix)

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.

Additional Items Required

- Coupling 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 sulfhydryl
- Wash Buffer: femtoTBST™ (Cat. # 786-161) or femtoPBST™ (Cat. # 786-162); 10X concentrated wash buffers supplemented with Tween® 20. Alternatively use 0.1M sodium phosphate, 0.15M NaCl and 0.05% Tween® 20 (pH7.4).
- Cysteine•HCl (Cat. # 786-713) to block unreacted maleimide sites
- Blocking Buffer: A suitable blocking buffer, we recommend *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, 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 1-50µg/ml in Coupling Buffer. Add up to 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 3 times with 200µl Wash Buffer.
5. Immediately prior to use, prepare a 10µg/ml cysteine solution and add 200µl to each well. Incubate for 1 hour at room temperature.
6. Continue with the ELISA or other assay.

APPENDIX

Ellman's Reagent (DTNB) Assay

1. Make 10mM DTNB stock solution by dissolving 40mg DTNB in 10ml 0.1M Tris-HCl pH 7.5. The stock solution can be stored at 4°C for 3 months. Dilute the stock solution 100 fold with 0.1M Tris-HCl pH 7.5 to make 0.1mM DTNB working solution.
2. Aliquot 950µl of 0.1mM DTNB work solution to each 1.5ml centrifuge tube. Add 50µl test sample and mix by brief vortexing. Set a blank by adding 50µl of 0.1M Tris-HCl pH 7.5 to 950µl of 0.1mM DTNB work solution.
   NOTE*: The test sample may need to be diluted before applied to the assay and the dilution factor should be recorded. The 50µl test sample applied to the assay reaction should have a sulfhydryl concentration less than 0.5mM. Concentrations exceeding 0.5mM free sulfhydryl will result in high absorbance values and less accurate estimation of the concentration based on the extinction coefficient.*
3. Incubate 2 minutes at room temperature.
4. Measure the absorbance of the test sample with a spectrophotometer against blank at 412nm.
5. Calculate the concentration of free sulfhydryls in the sample from the molar extinction coefficient of NTB
   (14.15 mM^{-1} cm^{-1}) as follow:
   mM free sulfhydryls = Absorbance / (path length x 14.15) x 20 x dilution factor
   Path length is the cuvette path length in centimeter (cm)
   20 is the dilution factor of 50µl sample to 1ml assay volume

Use of SATA to add Sulfhydryls

SATA (N-Succinimidyl S-Acetylthioacetate) (Cat. # BC96) introduce protected sulfhydryls into proteins, peptides and other molecules. It is a NHS esters of S-acetylthioacetic acid.

1. Immediately before reaction, dissolver ~7mg SATA in 0.5ml DMSO to give ~55mM solution.
2. Combine 1ml 2-10mg/ml protein solution in PBS with 10µl 55mM SATA.
3. Incubate at room temperature for 30 minutes
4. Desalt the solution with a desalting column equilibrated with PBS. We recommend G-Biosciences SpinOUT™ GT-600 (Cat. # 786-170).
5. Identify the fraction with the protein using absorbance at 280nm or a suitable assay.
6. Combine 1ml SATA-modified protein with 100µl 0.5M hydroxylamine, 25mM EDTA in PBS.
7. Incubate for 2 hours at room temperature.
8. Desalt as before using PBS supplemented with 10mM EDTA.

Use of Traut's Reagent to add Sulfhydryls

Traut's Reagent (2-Iminothiolane) (Cat. # BC95) is a cyclic thioimidate compound for thiolation of primary amines.

1. Dissolve the protein or peptide in a non-amine buffer at pH8.0. The addition of 2-5mM EDTA will prevent oxidation of generate sulfhydryls into disulfide bridges.
2. Add 2 to 20 fold molar excess of Traut's reagent to the protein solution.
   NOTE: *A 2mg/ml solution of Traut's reagent in water or buffer is a 14mM stock solution.*
3. Incubate the solution for 1 hour at room temperature.
4. Desalt the solution with a desalting column equilibrated with PBS with 2-5mM EDTA. We recommend G-Biosciences SpinOUT™ GT-600 (Cat. # 786-170).

RELATED PRODUCTS

Download our Assay Development Handbook.

http://info2.gbiosciences.com/complete-assay-development-handbook

For other related products, visit our website at www.GBiosciences.com or contact us.

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