Well-Coated™ Neutravidin™

96-well plates coated with Neutravidin™ for binding biotinylated molecules

INTRODUCTION

Well-Coated™ Neutravidin™ plates are designed to specifically bind biotinylated molecules, including biotin tagged antibodies, with minimal non-specific binding. This is particular advantageous for antibodies known to denature upon direct binding to polystyrene plates.

Biotin exhibits an extraordinary binding affinity for avidin ($K_a=10^{15}M^{-1}$) and Neutravidin™ ($K_a=10^{15}$ M^{-1}). Biotin and avidin interaction is rapid and once the bond is established it can survive up to 3M guanidine-hydrochloride and extremes of pH. Biotin-avidin bonds can only be reversed by denaturing the avidin protein molecule with 8M guanidine-hydrochloride at pH1.5 or by autoclaving. Neutravidin™ is in many respects is similar to avidin except that it has no carbohydrate side chains to eliminate lectin binding; is of near neutral pI (6.3) to reduce non-specific adsorption; lacks the RYD sequence eliminating interaction with RGD domain of adhesion receptors. The binding of Neutravidin™ is similar to that of avidin and streptavidin with less non-specific binding.

Well-Coated™ Neutravidin™ plates are suitable for direct, indirect, competitive and sandwich assays. 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
786-742	*Well-Coated*™ Neutravidin™ Coated 96 well plate	5 plates
786-743	*Well-Coated*™ Neutravidin™ Coated 8-well strip plate	5 plates
786-766	*Well-Coated*™ Neutravidin™ Coated 96 well plate, Black	5 plates
786-767	*Well-Coated*™ Neutravidin™ Coated 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*™ Neutravidin™:** ~15pmol D-biotin/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

- Biotinylated antibody (10µg/ml) to be bound to plate; visit www.GBiosciences.com for biotin labeling kits.
- 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.
- Blocking Buffer for dilution: 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).
- Antigen

- Enzyme Labeled Primary Antibody; visit www.GBiosciences.com for horseradish peroxidase (HRP) and alkaline phosphatase (AP) labeling kits.
- 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. Add 100µl biotinylated antibody to each well.
3. Incubate at room temperature for 1-2 hours, for optimal binding use a plate shaker.
4. Wash each well with 200µl Wash Buffer.
5. Make serial dilutions of the antigen, diluted in Blocking Buffer, and add 100µl to each well.
6. Incubate at room temperature for 0.5-1 hour with shaking.
7. Wash each well with 200µl Wash Buffer.
8. Add 100µl enzyme labeled primary antibody.
9. Incubate at room temperature for 0.5-1 hour with shaking.
10. Wash each well with 200µl Wash Buffer.
11. Detect the label signal according to the manufacturer's instructions

RELATED PRODUCTS

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

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