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INSTRUCTIONS

ProFoldin 96-well Protein-binding Plates

Protein binding plates

96-well Anionic Protein-binding Plate (black)
96-well Anionic Protein-binding Plate (transparent)
96-well Cationic Protein-binding Plate (black)
96-well Cationic Protein-binding Plate (transparent)

Catalog number

APP96B
APP96T
CPP96B
CPP96T

Introduction

The 96-well protein-binding plates can be used to study specific protein-protein, protein-nucleic acid or protein-ligand interactions. The Anionic Protein-binding Plates bind negatively charged proteins. The Cationic Protein-binding Plates bind positively charged proteins. Since the bound-proteins often maintain their native confirmation and their catalytic or binding activities, the bound protein can be recognized by a second molecule that specifically interacts with the bound protein. The second molecule can be a conformation-specific antibody, a protein, a peptide, a nucleic acid or a small molecule such as a receptor ligand, an enzyme inhibitor, or a drug molecule. The second molecule can be detected by biochemical or biophysical methods. The binding reactions in the 96-well plate format may be used for screening libraries of nucleic acids, peptides or drugs that specifically interact with the bound protein.

Protocols

- 1. Protein binding to the plate:** Add 200 μ l of 0.5 – 1 mg/ml protein solution in a low salt buffer (< 10 mM salt) in each well. Incubate at 4°C overnight. Then discard the solution and gently rinse the wells with 300 μ l of the low salt buffer twice.
- 2. Block non-specific binding:** Add 300 μ l of a blocker into each well. Incubate for 30 min. The following blockers are recommended for different second molecules that specifically interact with the bound protein:

The second molecule	Blocker
Blocker Buffer:	10 mM buffer, 10 mM NaCl, 0.01% Tween-20, 1 mM EDTA
Protein:	1 % BSA in Block Buffer
Nucleic acid:	1 x DNA-binding Blocker (catalog # NBP96N) in Block Buffer
- 3. Second molecule binding:** Discard the blocker and add 200 μ l of the solution with the second molecule in the Block Buffer. Incubate for 1 hr. Then discard the solution and rinse the wells with 300 μ l of the Blocker Buffer followed by 300 μ l of 10 mM TrisHCl, 10 mM NaCl. The concentration of the second molecule depends on the binding affinity to the bound protein. It can be 0.1 mg/ml for proteins, a lower concentration for an antibodies, a micromolar concentration for oligos or small molecules.
- 4. Detection of the second molecule:** Detect the second molecule by any biochemical or biophysical methods including enzyme activity if available or fluorescence or radioactivity signals if the second molecule is labeled. Nucleic acids can be detected by adding a fluorescence dye.