ProFoldin
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INSTRUCTIONS

ProFoldin
96-well Protein–binding Plates

96-well Anionic Protein-binding Plate (black)	**Catalog No. APP96B**
96-well Anionic Protein-binding Plate (transparent)	**Catalog No. APP96T**
96-well Cationic Protein-binding Plate (black)	**Catalog No. CPP96B**
96-well Cationic Protein-binding Plate (transparent)	**Catalog No. CPP96T**

Introduction

The 96-well protein-binding plates can be used to study specific protein-protein interactions, ELISA, protein-DNA or RNA 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 where the native conformation is required. The second molecule can be detected by biochemical, biophysical or immunological methods . The black background of the plate provides a minimum noise level for fluorescence detection of the second molecules. The transparent background of the plate provides an option of detection of the bound molecules based on light absorbance. The binding reactions in the 96-well plate format may be used for ELISA and studies of protein-protein, protein-peptide, protein-DNA or protein-RNA interactions.

Each plate set includes 4 plates.

Reference:

Sanjeevani Arora et al, Downregulation of XPF–ERCC1 enhances cisplatin efficacy in cancer cells, DNA Repair, Volume 9, Issue 7, Pages 745-753 (2010).

Sawanta A. et al, Role of mismatch repair proteins in the processing of cisplatininterstrand cross-links. DNA Repair 35: 126–136 (2015).

Protocols

1. **Protein binding to the plate:** Add 200 µl of 0.5 – 1 mg/ml protein solution in a low salt buffer (10 mM buffer, 10 mM NaCl, 0.01% Tween-20, 1 mM EDTA) in each well. Incubate the plate at 4°C overnight. Then discard the solution and gently rinse the wells with 300 µl of the low salt buffer.

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2. **Block non-specific binding:** Add 300 µl of a blocker into each well. Incubate for 30 min. Then discard the solution and gently rinse the wells with 300 µl of the low salt buffer. The following blockers are recommended for different second molecules that specifically interact with the bound protein:

The second molecule	**Blocker**
Protein:	1 % BSA in the low salt buffer
Nucleic acid:	1 x DNA-binding Blocker (catalog # NBP96N) in the low salt buffer

3. **Second molecule binding:** Add 200µl of the solution with the second molecule in the low salt buffer. Incubate for 1 hr. Then discard the solution and rinse the wells with 300 µl of the low salt buffer followed by 300 µl 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 antibodies or a micromolar concentration for DNA oligos.
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. For example, nucleic acids can be detected by adding 300µl of a diluted SYBR green solution and read fluorescence at 535 nm with excitation at 485 nm.

Relative products

NBP96T	96-well DNA–binding Plates (Transparent)
NBP96B	96-well DNA–binding plates (black)
NBP96N	Nucleic Acid Binding Blocker