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INSTRUCTIONS

ProFoldin
96-well DNA–binding Plates

96-well DNA-binding Plate (black) **Catalog No. NBP96B**
96-well DNA-binding Plate (transparent) **Catalog No. NBP96T**

Introduction

The 96-well DNA-binding plates are coated with positive charges that interact with nucleic acids, DNA or RNA. The plates can be used to study specific interactions between one nucleic acid and another or the interaction between a nucleic acid and a protein. First, the nucleic acid is bound to the plate by charge-charge interactions. After the plate is blocked, it is incubated with a second molecule, a nucleic acid or protein, that specifically interacts with the bound nucleic acid. The second molecule can be detected by biochemical or biophysical methods. The black plates are for fluorescence detection of the second molecules. The transparent plate s are for detection of the bound molecules using light absorbance. The binding reactions in the 96-well plate format may be used for screening libraries of nucleic acids or proteins that specifically interact with the bound nucleic acid. The DNA binding capacity is about 1.1 ng / mm^2 measured from a binding experiment using an oligo with a size of 1000 bases at a concentration of 2μg / μl. The bound DNA is stable at salt concentrations below 200 mM. The bound DNA is also stable in the presence of non-ionic detergents such as Triton X-100 and Brij-35 at concentrations below their CMC levels. SDS interferes with the DNA binding to the plates. Detergents may affect protein functions.

Each plate set includes 4 plates.

Protocols

1. **Nucleic acid binding to the plate:** Add 200 μl of 10 μg /ml DNA 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 for 2 hr to overnight[(a)]. Then discard the solution and gently rinse the wells with 300 μl of the low salt buffer.
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 nucleic acid:

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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 Tris-HCl, 10 mM NaCl. The concentration of the second molecule depends on the binding affinity to the bound nucleic acid. 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.

Publications

Arora S. 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).

Related products

NBP96N	Nucleic Acid Binding Blocker
APP96B	96-well Anionic Protein-binding Plates (black)
APP96T	96-well Anionic Protein-binding Plates (transparent)
CPP96B	96-well Cationic Protein-binding Plates (black)
CP96T	96-well Cationic Protein-binding Plates (transparent)
MPR020	Micro Phosphate Removal Column Set
NAR911	Nucleic Acid Removal Kit
PNR020	Protein and DNA Removal Spin-columns
CSF1	Cell Separation Filter Plate

For more information of molecular binding, separation and analysis, please visit www.profoldin.com.

Note: [(a)]. Overnight incubation at 4 °C helps more complete binding of the DNA to the plate.