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## INSTRUCTIONS

# ProFoldin 96-well DNA-binding Plates (Black)

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**Catalog Number**                      **NBP96B**

### Introduction

The DNA-binding plates are coated with cations that interact with nucleic acids which are anions. Since the binding is based on charge-charge interactions, the plates may also be used for RNA binding. If the bound DNA is a single-strand DNA, the bound DNA can specifically recognize its complementary strand by G-C and A-T base pairing. Therefore, the 96-well DNA-binding plates can be potentially used for studies of nucleic acid – nucleic acid interactions or nucleic acid – protein interactions. The black background of the plate provides a minimum noise level for fluorescence detection of the bound molecules.

The DNA binding capacity depends on the DNA size and concentration. For DNAs with a size of about 1000 bp at a concentration of  $2\mu\text{g} / \mu\text{l}$  is about  $1.1 \text{ ng} / \text{mm}^2$ . 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.

### Protocols

#### DNA – DNA interactions

The following protocol is based on the interaction between a 40-mer oligonucleotide (DNA1) and its complementary strand (DNA2). Since the DNA binding capacity and the DNA-DNA interactions depend on the DNA properties and concentrations, this protocol should be used as a reference only. If the DNA concentration is lower, a longer binding time should be considered. The experiment can be performed at 4°C or room temperature dependent on the stability and reactivity of the biomolecules.

- (1) **The first DNA binding:** Add 200  $\mu\text{l}$  of 0.5  $\mu\text{M}$  DNA1 per well. Incubate for 2 hr to overnight<sup>(a)</sup>.
- (2) **Block:** Discard the solution. Add 300  $\mu\text{l}$  of 1 X Nucleic Acid Binding Blocker (Catalog # NBP96N). Incubate for 1 hr.
- (3) **Rinse:** Discard the solution. Rinse each well three times with 300  $\mu\text{l}$  of 10 mM TrisHCl, 10 mM NaCl, pH 7.5.
- (4) **The second DNA binding:** Add 200  $\mu\text{l}$  of 0.5  $\mu\text{M}$  DNA2 per well. Incubate for 1 hr.
- (5) **Rinse:** Repeat Step (3).
- (6) **Detection:** Detect DNA2.

#### DNA – protein interactions

The recommended protocol for DNA – protein interaction is similar to that for DNA-DNA interaction shown above but the second DNA is a protein in the binding buffer in Step (4). It is recommended to use 1 % BSA to block the non-specific protein binding in Step (2).

Notes:

<sup>(a)</sup>. An overnight incubation at 4°C helps more complete binding of the DNA to the plate.