



**ProFoldin Protein Folding Services**  
290 Turnpike Road, Suite 6, Number 321  
Westborough, MA 01581-2843  
FAX: (508) 845-9258  
[www.profoldin.com](http://www.profoldin.com)  
[info@profoldin.com](mailto:info@profoldin.com)

## INSTRUCTIONS

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

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**Catalog Number**                      **NBP96T**

### 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 transparent background of the plate provides an option of detection of the bound molecules based on light absorbance.

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.