Product Number
829-07921

Quantity: 25 µL

Storage: -20°C

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IRDye® 700 p53 Consensus Oligonucleotide

p53 Consensus Oligonucleotide[1]

5′ -- TAC AGA ACA TGT CTA AGC ATG CTG GGG ACT --3′
3′ -- ATG TCT TGT ACA GAT TCG TAC GAC CCC TGA --5′

* Underlined nucleotides are the binding site

Introduction

Gel shift assays or electrophoretic mobility shift assays (EMSA) provide a simple method to study DNA-protein interactions. This assay is based on the principle that a DNA-protein complex will have a different mobility during electrophoresis than unbound DNA. These shifts can be visualized on a native acrylamide gel using labeled DNA to form the DNA-protein binding complex. The Aerius® and Odyssey® family of imaging systems offer a quick and easily adapted alternative method to radioisotopic and chemiluminescent detection methods for EMSA analysis and visualization.[2,3]

A DNA oligonucleotide end-labeled with IRDye 700 is a good substrate for protein binding. IRDye DNA detection is linear within a 50-fold dilution range from 9.1 fmol to 0.18 fmol. Additional benefits include no hazardous radioisotopes, no gel transfer to membrane or gel drying, no chemiluminescent substrate reagents, and no film exposure. Following electrophoresis, the gel can be imaged while in the glass plates. If necessary, the gel can be placed back in the electrophoresis unit and run longer.

Existing mobility shift assay protocols can be easily transformed into infrared assays by replacing the existing DNA oligonucleotides with IRDye oligonucleotides. The binding conditions and electrophoresis conditions will remain the same as with any other EMSA detection method.

Electrophoretic Mobility Shift Assay

A universal binding condition that applies to every protein-DNA interaction is not recommended, since binding conditions will be specific for each protein-DNA interaction. Thus, the user should establish the conditions of the binding reaction for each protein-DNA pair. Binding buffer should be the same for a specific DNA-protein complex as with any other mobility shift detection method used.

For IRDye 700 p53, the following binding reaction is a good starting point:

Reaction	µL
10X Binding Buffer (100 mM Tris, 500 mM KCl, 10 mM DTT; pH 7.5)	2
Poly (dI•dC) 1 µg/µL in 10 mM Tris, 1 mM EDTA; pH 7.5	1
25 mM DTT/2.5% Tween® 20	2
100 mM $MgCl_2$	1
Water	12
IRDye 700 p53	1
HeLa 4 hour Serum Response nuclear extract (Positive control) (5 µg/µL)	1
Total	**20**

After the addition of the DNA to the protein-buffer mix, reactions are incubated to allow protein binding to DNA. A typical incubation condition is 20-30 minutes at room temperature. Since IRDye infrared dyes are somewhat sensitive to light, it is best to keep binding reactions in the dark during incubation periods (e.g., put tubes into a drawer or simply cover the rack containing tubes with aluminum foil). After the incubation period, Orange Loading Dye 10X (LI-COR, P/N 927-10100) is added to the binding reaction for electrophoresis.

IMPORTANT: It is critical **NOT** to use any blue loading dye (e.g., Bromophenol blue), as this will be visible on the Odyssey® image. It is highly recommended that Orange Loading Dye 10X (LI-COR, P/N 927-10100) be used instead.

NOTE: In some cases, we observed that DNA control reactions (no protein) have lower signal than the reactions containing the protein. This may be due to lower stability of the dye in certain buffer conditions. The addition of a final concentration of 2.5 mM DTT and 0.25% Tween® 20 to all reactions reduces this phenomenon.

Gel electrophoresis of the DNA-protein complex is done using a polyacrylamide gel composed of Tris-acetate, Tris-borate, or Tris-glycine-EDTA gel and buffer at 10 V/cm at room temperature or at 4°C in the dark (simply put a cardboard box over the electrophoresis apparatus).

Storage

Store at -20°C protected from light; stable for 6 months from date of shipment.

References

1. Kastan, M.B., Zhan, Q., El-Deiry, W.S, Carrier, F., Jacks, T., Walsh, W.V., Plunkett, B.S., Vogelstein, B., and Fornace, A.J.Jr.. 1992. *Cell* 71:587-597.
2. Li, Y., F. Ahmed, S. Ali, P. A. Philip, O. Kucuk, and F. H. Sarkar, 2005. *Cancer Res.* 65:6934-6942.
3. Geddie, M. L., T. L. O'Loughlin, K. K. Woods, and I. Matsumura, 2005. *J. Biol. Chem.* 280: 35641-35646.