Product Number
829-07926

Quantity: 25 µL

Storage: -20°C

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LI-COR Biosciences

4647 Superior Street • P.O. Box 4000
Lincoln, Nebraska 68504 USA
North America: 800-645-4267
International: 402-467-0700
FAX: 402-467-0819

LI-COR GmbH Germany, Serving Europe, Middle East and Africa: +49 (0) 6172 17 17 771
LI-COR UK Ltd. UK, Serving UK, Ireland, and Scandinavia: +44 (0) 1223 422104
All other countries, contact LI-COR Biosciences or a local LI-COR distributor:
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IRDye® 700 Sp-1 Consensus Oligonucleotide

Sp-1 Consensus Oligonucleotide[1]

5′ -- ATT CGA TCG GGG CGG GGC GAG C -- 3′
3′ -- TAA GCT AGC CCC GCC CCG CTC G -- 5′

* Underlined nucleotides are the binding site

IRDye® 700 oligonucleotides are supplied as 25 µL of 50 nM (or 50 fmol/µL) double-stranded DNA.

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 Sp-1, 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
Water	13
IRDye 700 Sp-1	1
HeLa 4 hour Serum Response nuclear extract (Positive control) (5 µg/µL) in Dilution Buffer (20 mM Hepes (pH 7.9), 100 mM KCl, 1 mM $MgCl_2$, 20% glycerol, 0.5 mM PMSF and 0.5 mM DTT)	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. Lenardo, M.J., and Baltimore, D. 1989. *Cell* 58:227-229.
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.