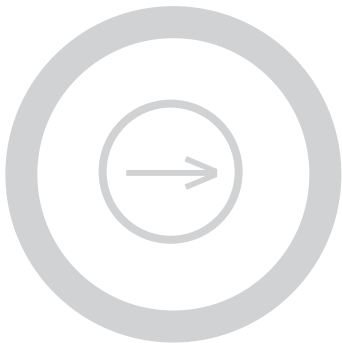


LI-COR IRDye[®]

Infrared Dye Reagents

ARE (Androgen Receptor) IRDye[®] 700 Infrared Dye Labeled Oligonucleotides

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LI-COR[®]

Biosciences

Doc #988-08524

I. ARE Consensus Oligonucleotide¹

5' -- GTC TGG TAC AGG GTG TTC TTT TT --3'

5' -- CAG ACC ATG TCC CAC AAG AAA AA --5'

* Underlined nucleotides are the binding site

IRDye[®] 700 Infrared Dye Labeled Oligonucleotides are supplied as 25 µl of 50 nM (or 50 fmole/ µl) double stranded DNA.

II. 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 un-bound DNA. These shifts can be visualized on a native acrylamide gel using labeled DNA to form the DNA-protein binding complex. The Odyssey[®] Infrared Imaging System (LI-COR Biosciences) offers 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 infrared dye labeled DNA detection is linear within a 50-fold dilution range from 9.1 fmol to 0.18 fmol. Additional benefits include no hazardous radioisotope, no gel transfer to membrane or gel drying, no chemiluminescent substrate reagents, and no film exposure. Following electrophoresis, the gel can be imaged while remaining in the glass plates. If necessary, the gel can be placed back into 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 infrared dye end-labeled oligonucleotides. The binding conditions and electrophoresis conditions will remain the same as with any other EMSA detection method.

III. Electrophoretic Mobility Shift Assay

A universal binding condition that would apply to every protein-DNA interaction cannot be 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.

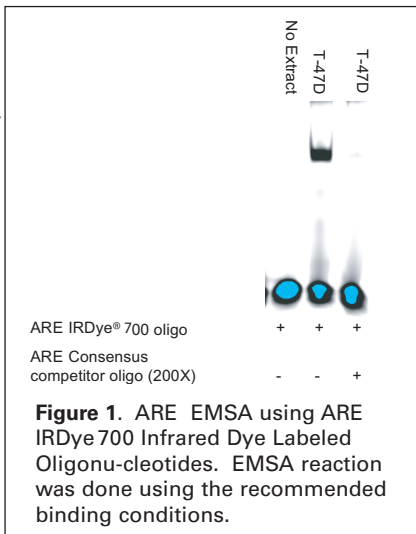
For ARE IRDye 700 Labeled Oligonucleotides, the following binding reaction is a good starting point:

| Reaction | µl |
|---|-----------|
| 10X Binding Buffer (100 mM Tris, 500 mM KC1, 10 mM DTT; pH 7.5) | 2 |
| Poly (dl-dC) 1 µg/µl in 10 mM Tris, 1 mM EDTA; pH7.5 | 1 |
| 25 mM DTT/2.5% Tween-20 | 2 |
| 100 mM MgCl ₂ | 1 |
| Water | 12 |
| ARE IRDye 700 Labeled Oligonucleotides | 1 |
| T-47D 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 IRDyes are somewhat sensitive to light, it is best to keep binding reactions during incubation periods in dark (e.g. put tubes into a drawer or simply cover the rack containing tubes with aluminum foil). After the incubation period, Orange Loading Dye (LI-COR, Part # 927-10100) is added to the binding reaction.

IV. Storage

Store at -20°C protected from light; stable for 1 year from date of shipment.



Continued

IMPORTANT: It is critical **NOT** to use any blue loading dye (Ex. bromophenol blue), as this will be visible on the Odyssey image. It is highly recommended that Orange Loading Dye (LI-COR, Part # 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 4% polyacrylamide gel composed of Tris-acetate, Tris-borate, or Tris-glycine-EDTA gel and buffer at 10 V/cm at room temperature at 4°C in the dark.

For best results, electrophoresis should be performed in dark (simply put a cardboard box over the electrophoresis apparatus).

V. References

1. Roche, P. J., S. A. Hoare, and M. G. Parker, 1992. *Mol. Endocrinol.* 6:2229-2235.
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

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