

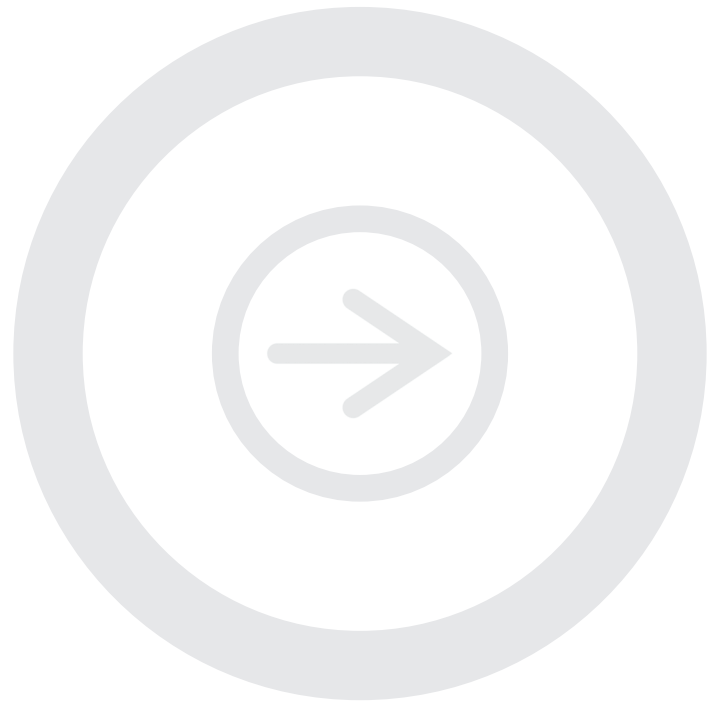
Odyssey[®]

Infrared Imaging System

NF κ B IRDye[™] 700 Infrared Dye Labeled Oligonucleotides

Published October, 2004. The most recent
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Doc# 988-07764



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I. NFκB Consensus Oligonucleotide¹

5' -- AGT TGA GGG GAC TTT CCC AGG C -- 3'
3' -- TCA ACT CCC CTG AAA GGG TCC G -- 5'

* Underlined nucleotides are the binding site.

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.

A DNA oligonucleotide end-labeled with IRDye™ 700 infrared dye 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 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™ 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.

For NFκB IRDye™ 700 infrared dye labeled oligonucleotides the following binding reaction is a good starting point:

Reaction	μl
10 X Binding Buffer (100mM Tris, 500mM KCl, 10mM DTT; pH 7.5)	2
Poly(dI•dC) 1μg/μl in 10mM Tris, 1mM EDTA; pH7.5	1
25mM DTT/2.5%Tween®-20	2
Water	13
NFκB IRDye™ 700 Infrared Dye Labeled Oligonucleotides	1
Raji nuclear extract (Positive control) (5μg/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™ 700 infrared dye is 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 cover the tube rack with aluminum foil). After the incubation period, 1X Orange Loading Dye (LI-COR, Part # 927-10100) is added to the binding reaction for electrophoresis.



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 protein. This may be due to lower stability of the dye in certain buffer conditions. The addition of 2.5mM DTT and 0.25% Tween® 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.

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

IV. Storage

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

V. References

1. Lenardo, M.J., and Baltimore, D. 1989. Cell 58:227-229.

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