Odyssey®

Infrared Imaging System

Active Motif TransAM™ Transcription Factor Assays on the Odyssey® System

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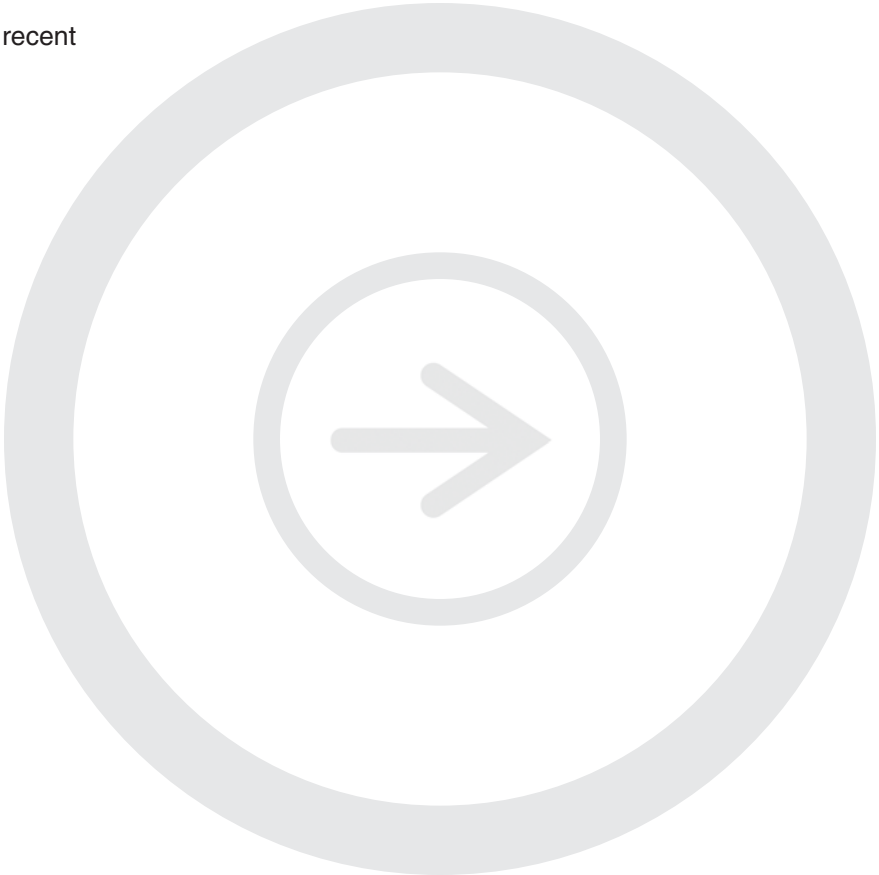

LI-COR® Biosciences

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I. Introduction

Active Motif (Carlsbad, CA) offers a colorimetric/chemiluminescent ELISA based assay kit for transcription factor measurement. The assay is done in 96-well format broken down into 8-well strips. A wide range of assay kits are available (AP-1, p53, NF_B, CREB, etc.). For more information about the contents and availability of these assays, please contact Active Motif at 1-877-222-9543 or www.activemotif.com. A specific oligonucleotide corresponding to the kit target protein has been immobilized to the bottom of a 96-well plate. Nuclear extract is bound to the DNA oligo followed by primary antibody incubation. To detect the protein of interest, a secondary HRP-conjugated antibody is applied and an HRP substrate is used. This gives a colorimetric/luminescent change that is measured. According to the protocol this takes less than five hours to complete. The nature of this assay makes it very amenable for use on the Odyssey®. A simple change from an HRP-conjugated secondary antibody to IRDye™-conjugated secondary antibody allows the assay to be converted for direct detection with Odyssey. This small change eliminates the enzymatic reaction steps in the protocol and reduces the amount of time needed to do the assay. It also eliminates the need for stopping the colorimetric reaction. The assay is easily completed in 3.5 hours. Dry assay plates can be stored in the dark and scanned at any time without compromising results.

II. Required Reagents

- TransAM™ Transcription Factor Assay Kit (Active Motif, Carlsbad, CA)
- IRDye™ 800CW- conjugated antibody (Rockland Immunochemicals, Gilbertville, PA) which corresponds to the HRP-conjugated antibody provided in the TransAM™ Transcription Factor Assay Kit.

III. General Guidelines for Converting the Colorimetric or Chemiluminescent Assay to an Infrared Assay

Binding of the Transcription Factor to the Consensus Sequence

Follow the protocol provided in the TransAM™ instruction manual.

Binding of the Primary Antibody

Follow the protocol provided in the TransAM™ instruction manual.

Binding of the Secondary Antibody

1.	Add 100 µl of diluted IRDye™ 800CW conjugated secondary antibody (1:1000 dilution in 1X Antibody Binding Buffer) to all wells being assayed.
2.	Cover the plate and incubate in the dark for 1 hour at room temperature without agitation.
3.	Wash the wells 4 times with 200 µl 1X washing buffer (provided in kit).

Detection

1.	Place plate on the front left corner of Odyssey scanning surface. Scan the 800 channel using an initial intensity setting of 8, a resolution of 169 µm, and focus offset of 3 mm. If your image signal is saturated or too high, re-scan using a lower intensity setting. If your image signal is too low, re-scan using a higher intensity setting. •

IV. Experimental Results

A TransAM™ p53 Assay Kit (Active Motif, Cat # 41196) was used with Raji nuclear extract dilutions of 15, 12.5, 10, 7.5, 5, 2.5, 1.25, and 0 µg. The protocol above was followed. Comparisons were made using complete binding buffer and complete binding buffer with free wild-type consensus oligonucleotide added to compete for binding (Figure 1.)

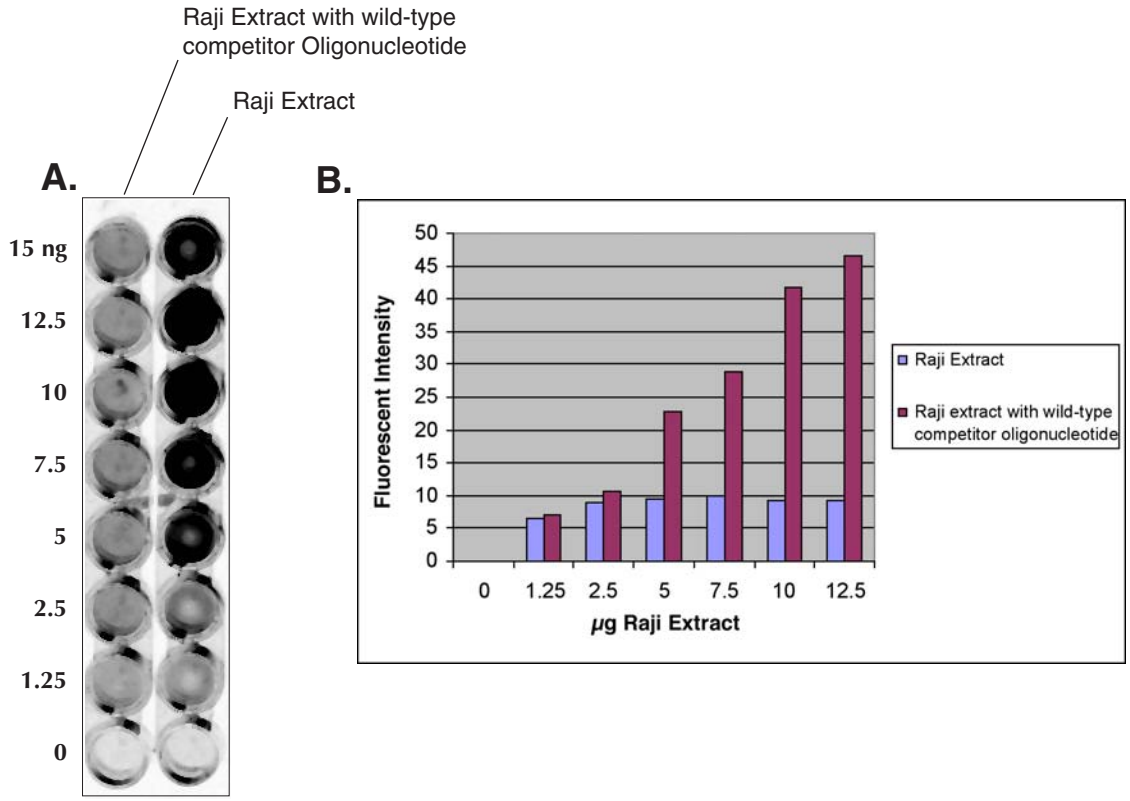

Figure 1. p53 TransAM data. Assay was done according to manufacturer's protocol replacing the secondary HRP-antibody with a secondary IRDye™ 800CW antibody. A.) Odyssey image of the assay. Row 1 shows Raji nuclear extracts at dilutions of 15, 12.5, 10, 7.5, 5, 2.5, 1.25, and 0 µg of extract using complete binding buffer with free wild type consensus oligonucleotide. Row 2 shows Raji nuclear extracts of the same dilutions using complete binding buffer without competitor. B.) Data chart following negative control subtraction as background.

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