

# Odyssey<sup>®</sup>

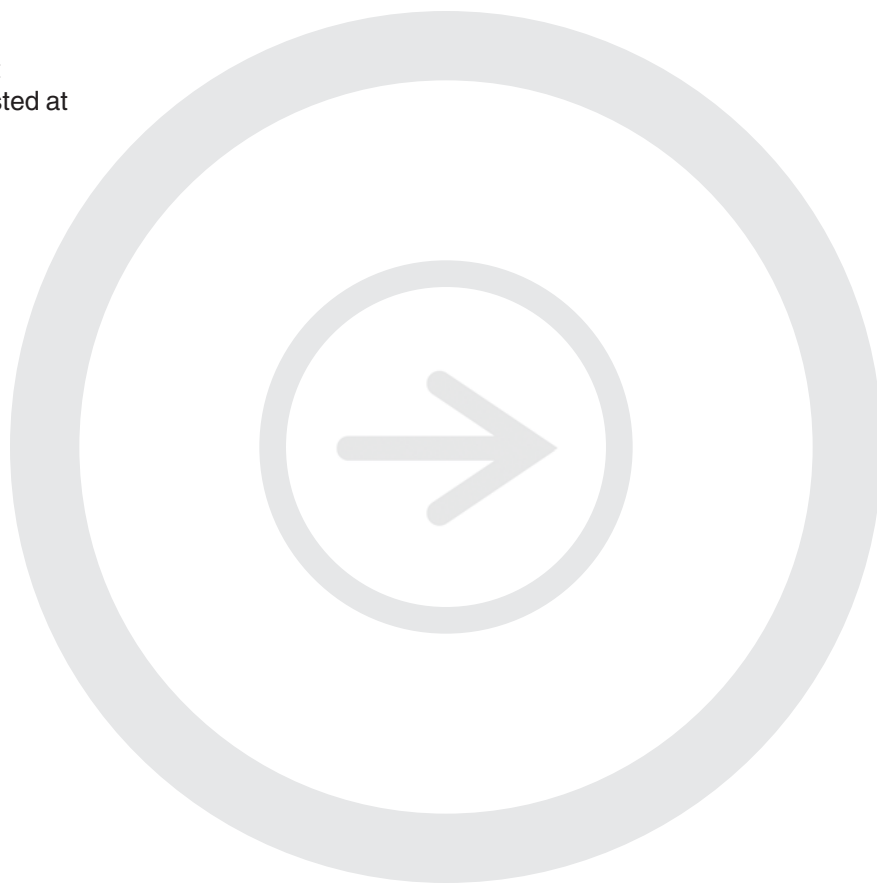
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Infrared Imaging System

## **Cayman Chemical\* PPAR $\gamma$ Transcription Factor Kit Assay on the Odyssey System**

**\* Cayman Chemical, Ann Arbor, MI**

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## I. Introduction

The Cayman Chemical (Ann Arbor, MI) PPAR $\gamma$  Transcription Factor Assay is a 96-well format ELISA-based kit for nonradioactive detection of DNA binding activity in nuclear extracts. Convenient 8-well strips are precoated with double-stranded DNA containing the peroxisome proliferator response element (PPRE) specific for human peroxisome proliferator-activated receptors (PPAR) subtype  $\gamma$ . There is no cross-reactivity with the other PPAR subtypes  $\sigma$  or  $\alpha$ . The PPARs in nuclear extracts bind the PPRE element and are detected using a primary antibody specific to PPAR $\gamma$ . Binding of the secondary HRP-labeled anti-species conjugated antibody provides for colorimetric detection at 450nm. The kit includes buffer recipes and a protocol for purification of cellular nuclear extracts. Competitor DNA is provided as a means to confirm assay specificity. The employment of an HRP-conjugated detection antibody facilitates the conversion of the assay to a direct detection method using the Odyssey<sup>®</sup> system by substitution of IRDye<sup>®</sup>-labeled secondary antibody. This simple change reduces the amount of time to complete the assay and eliminates the need for additional reagents to stop the reaction. Dry assay plates can be stored in the dark and scanned at any time without compromising results.

## II. Required Reagents

- PPAR $\gamma$  Transcription Factor Assay Kit (Cayman Chemical, Cat. #10006855).
- IRDye 800CW-conjugated antibody (LI-COR Biosciences) which corresponds to the HRP-conjugated antibody provided in the Transcription Factor Assay Kit. For the PPAR $\gamma$  kit use IRDye 800CW Goat Anti-Rabbit labeled secondary antibody (LI-COR Biosciences Cat. #926-32211).
- Odyssey Blocking Buffer containing 0.2% Tween<sup>®</sup>-20 (LI-COR Biosciences, Cat, #927-40000).

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

### Binding of the Transcription Factor to the Consensus Sequence

Follow the protocol provided in the PPAR $\gamma$  instruction manual.

### Binding of the Primary Antibody

Follow the protocol provided in the PPAR $\gamma$  instruction manual.

### Binding of the Secondary Antibody

1. Add 100  $\mu$ l of diluted IRDye 800CW-conjugated secondary antibody (1:1000 dilution in Odyssey blocking buffer containing 0.2% Tween-20) to all wells being assayed except blank wells.
2. Use the adhesive cover provided to seal the plate and incubate in the dark at room temperature for 1 hour without agitation.
3. Wash the wells 5 times with 200  $\mu$ l of 1X wash buffer (provided in kit).
4. Open the Odyssey cover, remove the strips from the plate carrier and place directly on the front left corner of Odyssey scanning surface. Scan in the 800 channel using an initial intensity setting of 10, a resolution of

169  $\mu\text{m}$ , and focus offset of 1.5 mm. If the image signal is saturated, or too high, re-scan using a lower intensity setting. If the image signal is too low re-scan using a higher intensity setting.

## IV. Experimental Results

Increasing amounts of positive control lysates supplied with the PPAR $\gamma$  Transcription Factor Assay Kit were assayed for PPAR $\gamma$  binding activity comparing the kit supplied HRP conjugated antibody to the IRDye 800CW conjugated secondary antibody (Figure 1A). A separate assay was performed in the presence of competitive ds DNA to confirm the specificity of the assay (Figure 1B). A reduction in signal correlates with binding of the competitive DNA rather than PPAR. The protocol above was followed. The data for the HRP detection method was collected using a Spectramax plate reader at 450 nm. The wells receiving the IRDye 800CW was scanned at intensity 10, 169  $\mu\text{m}$  resolution and with a focus offset of 1.5 mm. The strip wells were placed directly on the scanner surface.

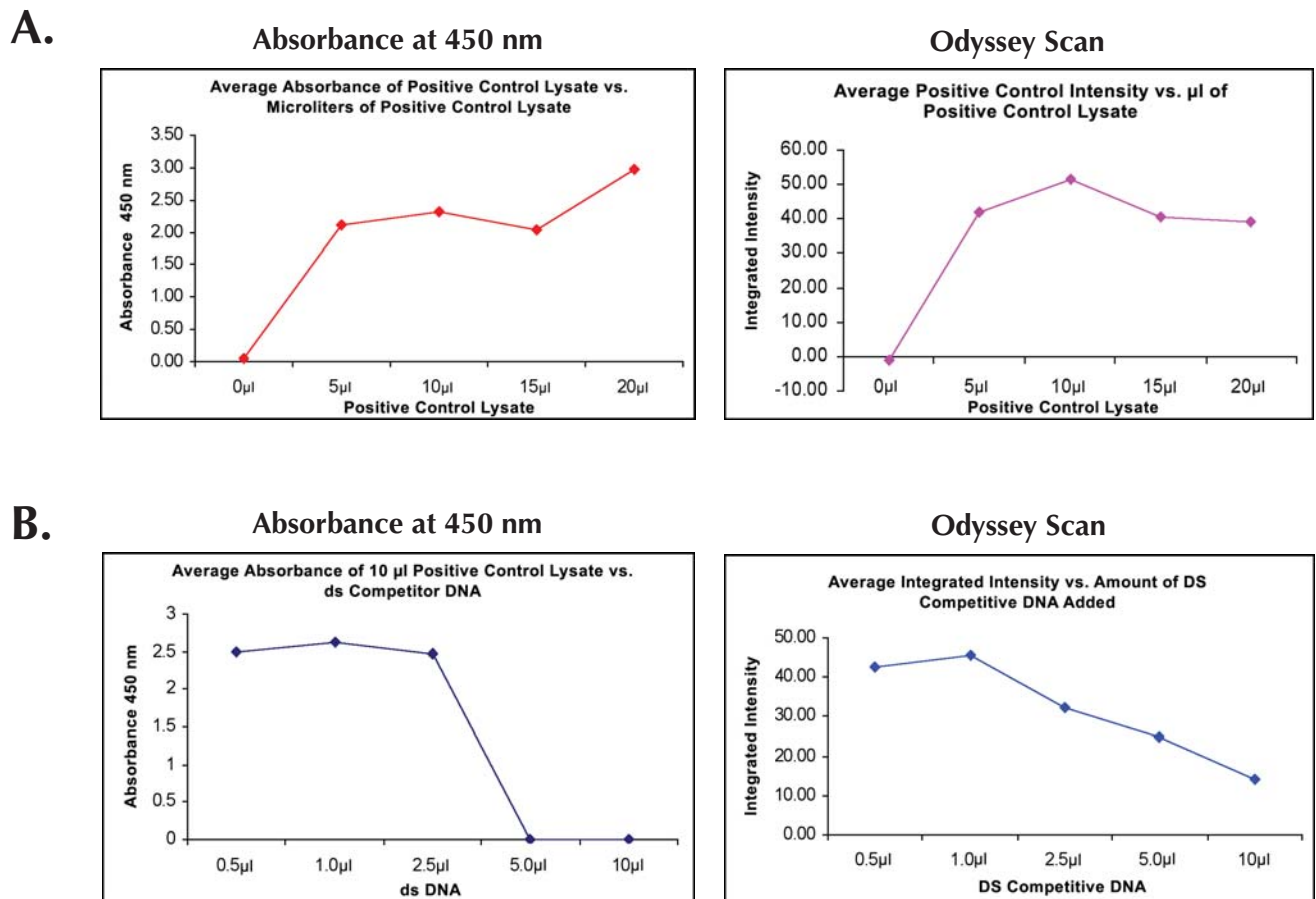


Figure 1. PPAR $\gamma$  Data. A.) Comparison of the data using increasing amounts (0, 5, 10, 15, and 20  $\mu\text{l}$ ) of positive control lysate in complete binding buffer and either the HRP conjugated antibody or IRDye 800CW conjugated antibody for detection. B.) Comparison of reduction in signal with increasing amounts of added competitive DNA. Note that the increased sensitivity of the assay using the IRDye 800CW results in a more gradual drop-off in the signal with higher amounts of competitor, while the HRP detection signal drops sharply to 0 at both 5 and 10  $\mu\text{l}$  of DNA.

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