

# IRDYE®

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Infrared Dye Reagents

## **RepliGen\* Protein A ELISA Assay on the Odyssey® System**

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## I. Introduction

RepliGen Corporation offers a colorimetric ELISA-based assay kit for the detection of Protein A in the presence of murine or human antibodies. The assay is done in 96-well format with removable 8-well strips. For more information about the contents and availability of these assays, please contact RepliGen Corporation at 1-800-622-2259 or [www.repligen.com](http://www.repligen.com).

The microtiter wells provided in the kit are coated with anti-Protein A antibodies. Samples and Protein A standards are diluted with sample diluent, and incubated with the solid-phase antibodies. Captured Protein A is then detected by the addition of a biotinylated rabbit anti-Protein A probe, followed by a Streptavidin Peroxidase conjugate.

The final detection step involves the use of TMB and 1N Phosphoric Acid to give a highly sensitive colorimetric reaction. The nature of this assay makes it very amenable for use on the Odyssey®. A simple substitution of the Streptavidin Peroxidase conjugate with IRDye® 800CW Streptavidin allows the assay to be converted for direct detection with Odyssey. This small change eliminates the enzymatic reaction and the need for stopping the colorimetric reaction with Phosphoric Acid. The assay is easily completed in 2.5 hours. Dry assay plates can be stored in the dark and imaged at a later time without compromising results.

## II. Required Reagents

- Protein A ELISA Kit (RepliGen Corporation, Part No. 9000-1)
- IRDye 800CW Streptavidin (LI-COR Biosciences, Part No. 926-32230), which replaces the Streptavidin Peroxidase Conjugate provided in the Protein A ELISA Kit

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

### ***Preparation of Standard and Samples to be assayed***

Follow the protocol provided in the Protein A ELISA Kit User Manual

### ***Binding of the Primary Antibody (biotinylated anti-Protein A)***

Follow the protocol provided in the Protein A ELISA Kit User Manual

### **Binding of the Streptavidin**

1. Add 12  $\mu\text{l}$  IRDye 800CW Streptavidin (1mg/ml) to 12 ml PBS-Tween (provided in kit). Mix well by inverting tube several times.
2. Add 100  $\mu\text{l}$  of diluted IRDye 800CW Streptavidin to all wells, including substrate-only control wells. Cover the plate with sealing foil and incubate, protected from light, for 30 minutes at room temperature.
3. Wash the wells 2 times with PBS-Tween and then 2 times with PBS (both provided in kit). Pour the PBS off the wells and dry the wells by tapping the inverted plate on clean paper towels.

### **Detection**

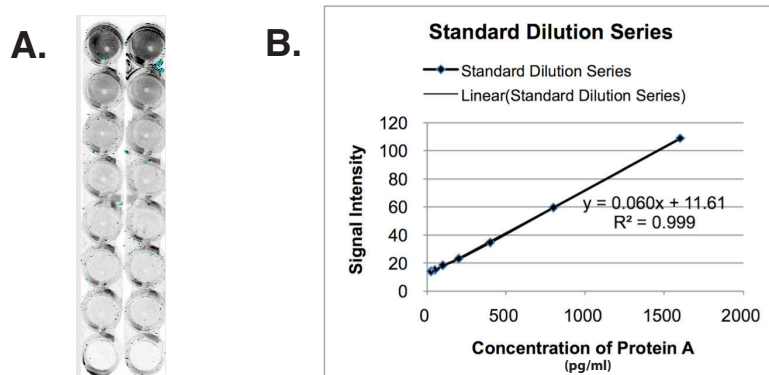
1. Remove the strips from the 96-well frame and place directly on the front left corner of the Odyssey scanning surface.

*NOTE: If the strips are left in the frame, they will not image correctly due to the distance from the imaging surface.*

2. Scan the 800 channel using an Intensity setting equal to 10, a resolution of 169  $\mu\text{m}$ , and focus offset of 1.1 mm.

## **IV. Experimental Results**

A Protein A ELISA Kit Standard Dilution series was assayed using the protocol outlined.



**Figure 1.** Protein A Standard was assayed according to manufacturer's protocol replacing the Streptavidin Peroxidase Conjugate with IRDye 800CW Streptavidin.

- A.) Odyssey image of the replicated standards assayed in duplicate.
- B.) Graph of Standard dilution data series displayed as concentration of Protein A vs. Signal Intensity.

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