

Product Number

926-34301

See storage recommendations
for each component

Revised: September 2011

Updates available at:

<http://biosupport.licor.com>

LI-COR DOES NOT PROVIDE RESEARCH ADVICE OR DETERMINE OR RECOMMEND ANY POTENTIAL USES FOR IRDYE INFRARED DYES AND REAGENTS. LI-COR MAKES NO WARRANTIES OF ANY KIND, EITHER EXPRESS OR IMPLIED, AS TO ANY MATTER INCLUDING, BUT NOT LIMITED TO, WARRANTY OF FITNESS FOR PURPOSE, OR MERCHANTABILITY OR RESULTS OBTAINED FROM USE OF IRDYE INFRARED DYES AND REAGENTS. IN NO EVENT SHALL LI-COR BE LIABLE FOR LOST PROFITS, CONSEQUENTIAL, EXEMPLARY, SPECIAL, DIRECT, INCIDENTAL, OR PUNITIVE DAMAGES, OR ATTORNEY FEES, EVEN IF LI-COR HAD BEEN ADVISED OF, KNEW OR SHOULD HAVE KNOWN, OF THE POSSIBILITIES THEREOF. NO EMPLOYEE, AGENT OR REPRESENTATIVE OF LI-COR HAS THE AUTHORITY TO BIND LI-COR TO ANY ORAL REPRESENTATION OR WARRANTY EXCEPT AS SPECIFICALLY SET FORTH HEREIN.

You, the Buyer/User, agree to abide by and observe LI-COR's Standard Terms and Conditions of Sale, which are available on the internet at <http://www.licor.com/corp/terms.jsp> or upon request.

© 2011 LI-COR, Inc. LI-COR is an ISO 9001 registered company. IRDye reagents, and the Aerius and Odyssey imaging systems are covered by U.S. and foreign patents and patents pending. LI-COR, IRDye, and Odyssey are registered trademarks of LI-COR, Inc. in the United States and other countries. All other trademarks belong to their respective owners.

Doc #988-12460

LI-COR

Biosciences

4647 Superior Street • P.O. Box 4000
Lincoln, Nebraska 68504 USA
North America: 800-645-4267
International: 402-467-0700
FAX: 402-467-0819

LI-COR GmbH Germany, Serving Europe,
Middle East and Africa: +49 (0) 6172 17 17 771
LI-COR UK Ltd. UK, Serving UK, Ireland and
Scandinavia: +44 (0) 1223 422104
All other countries, contact LI-COR Biosciences
or a local LI-COR distributor;
<http://www.licor.com/distributors>

www.licor.com/bio

ELISA AP Substrate (680) Pack

Applications

Substrate selection is a key component in developing ELISAs with high signal and low background. LI-COR Biosciences offers proprietary HRP 680 and AP 680 Substrates that are optimized for use in the near-infrared region (680 nm) on the Aerius, and Odyssey® family of imaging systems. Near-infrared (NIR) detection overcomes the limitations of chromogenic substrate detection, which does not allow for quantification of greater than four optical density units. NIR detection allows for a wider linear range.

The 680 substrates offer equal or better sensitivity compared to commercially available chromogenic substrates and are ideal for endpoint assays. AP 680 Substrate results in a colorless-to-light-blue product in the presence of alkaline phosphatase. The reaction is stopped with a colored AP 680 Stop Solution. The substrate provides excellent signal-to-noise ratios, consistency, and linearity, making it an ideal substrate for use in most ELISA applications. *This product is not intended for use in Western blotting applications.*

Components

AP 680 Substrate, 1 vial (substrate)

AP 680 Assay Buffer, 110 mL (assay buffer)

AP 680 Stop Solution, 30 mL (stop solution)

Storage and Stability

AP 680 Substrate. Protect from light. Store substrate at -20°C prior to reconstitution.

The lyophilized substrate is stable for 6 months from date of receipt.

AP 680 Assay Buffer. Store assay buffer at $2 - 8^{\circ}\text{C}$. The assay buffer is stable for 6 months from date of receipt.

AP 680 Stop Solution. Store stop solution at $2 - 8^{\circ}\text{C}$. The stop solution is stable for 6 months from date of receipt.

Directions for Use

Prepare concentrated stock solution of AP 680 Substrate by reconstituting contents of vial with 1.0 mL sterile ultrapure water. Vortex thoroughly for one minute. Store concentrated stock solution at $2 - 8^{\circ}\text{C}$, protected from light. The concentrated stock solution is stable for 3 months from date of reconstitution, when stored as indicated.

Prepare AP 680 Substrate working stock solution, immediately prior to use, by adding 100 μL of concentrated stock solution in 10 mL of AP 680 Assay Buffer. This is enough material for one 96-well microplate. Discard unused portion of working stock solution.

Guidelines for Optimal ELISA Performance with Near-Infrared Detection

Many commercially-available microwell plates and strip wells designed for ELISA or EIA/RIA use are compatible with the Aerius, and Odyssey family of imaging systems. **Clear, flat-bottomed plates are a requirement.** Use of non-clear-bottom plates will result in no signal. It is advisable to scan the plate prior to addition of substrate to ensure the plate does not fluoresce in the 700 nm channel.

Required Materials

- 96-well ELISA plate or strip well that has been detected with an alkaline phosphatase secondary antibody or alkaline phosphatase streptavidin
- Calibrated multichannel pipette and appropriate tips
- Thermo Scientific® Matrix Reagent reservoirs, part number 14-387-069 (recommended for use with small volumes) or equivalent
- AP 680 Substrate (working stock solution)
- AP 680 Stop Solution (stop solution)
- Plate sealer or lid
- Odyssey®, Odyssey Sa, or Aerius Imaging System

Procedure

A. Addition of Substrate and Stop Solution

1. Allow the substrate working stock solution to equilibrate to room temperature, protected from light, prior to use.
2. Following incubation with alkaline phosphatase labeled conjugate, wash the plate using normal wash methods and blot dry by inverting the plate and tapping gently on clean, dry paper towels.
3. Add **100 µL** substrate working stock solution to each well. Seal plate or replace lid and incubate for desired length of time, at room temperature, with gentle shaking. Protect from light. *NOTE: Length of incubation time is dependent upon assay. It is recommended to start with a 10 - 15 minute incubation time and adjust accordingly to achieve optimal signal-to-noise ratios.*
4. Remove 3 mL of AP 680 Stop Solution from bottle. This will be enough for one 96-well microplate. Allow 3 mL aliquot to equilibrate to room temperature prior to use. *NOTE: It is important to use a reservoir designed specifically for small volumes (recommended in required materials) to conserve the amount of AP 680 Stop Solution used for each plate (3 mL).*
5. Add **25 µL** Stop Solution to each well. Seal or replace lid. Incubate for 5 minutes, with gentle shaking, at room temperature. Protect from light. All of the wells with Stop Solution will appear purple. The color change does not affect quantification.
6. Wipe the bottom of the plate gently with a clean, lint-free wipe to remove dust or dried liquid, as these will fluoresce in the 700 nm channel and possibly affect quantification.
7. Scan the microwell plate. Please refer to your manual for specific information on your imager model.

B. Sample Quantification (applicable for the Aerius, and Odyssey family of imaging software)

1. Quantification is performed by applying a grid to the microwell plate image (choose **Analyze > Add Grid**).
2. If none of the default Grid Templates fit your microwell plate, use the Grid Template settings (choose **Settings > Grid Templates**) to modify grid parameters. Adjust the well diameter and other parameters to correspond with the microwell plate you are using.
3. Quantification data can be viewed in the grid sheet (choose **Analyze > Grid Sheet**) or by using the Report tab (choose **Report > Report view**). If specks interfere with quantification, it is recommended to use trimmed mean values instead of Int. Intensity values. Trimmed mean can be selected by editing the report fields in the template. Consult the User Guide for your system for more detailed microwell plate quantification procedures.

C. Hints and Tips

If using the Odyssey, it is beneficial to clean the scan bed thoroughly with 100% methanol. Spray scan bed surface with canned air to eliminate lint or dust which appears as specks in the image and may interfere with quantification.