Product Number
926-34300

See storage recommendations for each component

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ELISA HRP 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. HRP 680 Substrate results in a colorless-to-light-blue product in the presence of horseradish peroxidase. The reaction is stopped with a colored HRP 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

HRP 680 Substrate, 1 vial (substrate)
HRP 680 Assay Buffer, 110 mL (assay buffer)
HRP 680 Stop Solution, 30 mL (stop solution)

Storage and Stability

HRP 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.

HRP 680 Assay Buffer. Store assay buffer at 2 – 8°C. The assay buffer is stable for 6 months from date of receipt.

HRP 680 Stop Solution. Store stop solution at 2 – 8°C. The stop solution is stable for 6 months from date of receipt.

Directions for Use

Prepare concentrated stock solution of HRP 680 Substrate by reconstituting contents of vial with 1.0 mL ethanol (ethyl alcohol). Vortex thoroughly for one minute. Store concentrated stock solution at -20°C, protected from light. The concentrated stock solution is stable for 3 months from date of reconstitution, when stored as indicated.

Prepare HRP 680 Substrate working stock solution immediately prior to use, by adding 100 µL of concentrated stock solution in 10 mL of HRP 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 required**. 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.

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Required Materials

- 96-well ELISA plate or strip well that has been detected with a horseradish peroxidase secondary antibody or horseradish peroxidase streptavidin
- Calibrated multichannel pipette and appropriate tips
- Thermo Scientific® Matrix Reagent reservoirs, P/N 14-387-069 (recommended for use with small volumes), or equivalent
- HRP 680 Substrate (working stock solution)
- HRP 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 HRP-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 HRP 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 HRP 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 light pink. 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 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 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.