Product Information

SIGMA*FAST*™ OPD

Tablet set

P9187

Product Description

The SIGMA*FAST*™ OPD (*o*-phenylenediamine dihydrochloride) tablet set is designed for use as a soluble substrate to detect horseradish peroxidase (HRP) activity in Enzyme Immunoassays (EIA and ELISA).[1-3] EIA/ELISA applications utilizing OPD may be read in timed assays or stopped with dilute acid solutions for delayed readings. The SIGMA*FAST*™ OPD tablet set requires only the addition of water, with no additional buffers or steps, to prepare an active substrate solution.

Several dissertations[4-9] have cited use of product P9187 in their research protocols.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Components

Each SIGMA*FAST*™ OPD tablet set consists of one OPD tablet (silver foil) and one urea hydrogen peroxide tablet (gold foil). The tablets are individually packaged in foil packets.

Storage/Stability

Store the tablet sets at 2-8 °C. The tablets are very hygroscopic and light-sensitive, and should be stored in the protective foil packets.

The Substrate Solution is light-sensitive and should be protected from direct sunlight or UV sources in a tightly capped amber bottle.

Preparation Instructions

Each tablet set, when dissolved in 20 mL of water, provides 20 mL of ready-to-use substrate with final concentrations of:

- 0.4 mg/mL OPD
- 0.4 mg/mL urea hydrogen peroxide
- 0.05 M phosphate-citrate, pH 5.0

1. Remove the required number of OPD and urea hydrogen peroxide tablets for the assay.
2. Return the box to the refrigerator.
3. Allow the tablets to reach room temperature.
4. Open one OPD tablet package (silver foil) and one urea hydrogen peroxide tablet package (gold foil).
5. Drop the tablets into an amber bottle containing 20 mL of water. **Avoid skin contact with the tablets**.
6. Vortex until dissolved.

The Substrate Solution is now ready for use. For best results, the Substrate Solution should be used within one hour.

Procedure

1. After the EIA/ELISA reaction with the HRP-conjugated antibody is completed, wash the plate thoroughly to remove unbound conjugate.
2. Add 200 µL of the Substrate Solution to each well. Incubate the plate, in the dark, for 30 minutes at room temperature.
3. After the incubation period, read the plate at 450 nm on a multiwell plate reader.
4. If the plate cannot be read immediately, add 50 µL of 3 M HCl or 3 M H_2SO_4 solution per 200 µL of solution. Read stopped reactions at 492 nm.

Troubleshooting

If the background is too high:

1. Use a blocking step prior to the application of the primary antibody. Normal serum (5% v/v) from the same species as the host of the second antibody generally produces the best results.
2. Additional blocking agents for an ELISA are:
   - 0.05% TWEEN® 20 in 50 mM TBS, pH 8.0 (Cat. No. T9039).
   - 1% BSA containing 0.05% TWEEN® 20 in 50 mM TBS, pH 8.0.
   - 3% nonfat-dried milk in 0.01 M TBS (Cat. No. P2194). Do not use milk as a blocking agent when using avidin-biotin systems.
3. Use 0.05% TWEEN® 20 in all washing and antibody diluent buffers.
4. Run control wells without the primary antibody to check for nonspecific reactivity of the secondary antibody.
5. Titer the primary antibody and the conjugate to optimize working dilutions.

If no color develops or the color is too faint:

1. Adjust the concentration of the primary antibody.
2. Adjust the concentration of the secondary antibody.
3. Determine if the enzyme conjugate is active by mixing a small sample of substrate and conjugate together in a tube.
4. Increase the reaction time or temperature.
5. Adjust the concentration of the coating antigen.
6. Consider using an amplifying system such as avidin-biotin.

References

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