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Product Information

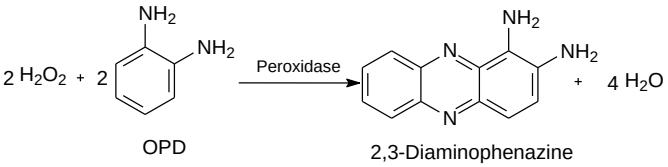
o-Phenylenediamine
tablet, 20 mg substrate per tablet

Catalog Number **P5412**
Storage Temperature 2–8 °C

CAS RN 95-54-5
Synonyms: 1,2-Benzenediamine,[1] OPD

Product Description

o-Phenylenediamine (OPD) is a chromogenic substrate suitable for use in ELISA procedures which utilize horseradish peroxidase conjugates.[2,3] This substrate produces a soluble end product that is orange-brown in color and can be read spectrophotometrically at 450 nm. The OPD reaction may be stopped with 3 M HCl or 3 M H_2SO_4, and read at 492 nm.



OPD 2,3-Diaminophenazine

The oxidation product of *o*-phenylenediamine produced by horseradish peroxidase is 2,3-diaminophenazine. This product has been characterized by melting point, mass spectrometry, and NMR.[4,5]

Various studies have cited use of this product.[6-12]

This product is supplied as 50 or 100 tablets per box, individually foil wrapped for ease of use, storage, and safety. Each tablet weighs ~45 mg (range 40–50 mg) and contains 20 mg of substrate. One tablet, dissolved in 10 mL of water, gives a solution with a pH of 9.0 (range 8.5–9.5). The background absorbance of this solution cannot be more than 0.04.

Precautions and Disclaimer

This product is 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.

Storage/Stability

Store tablets at 2–8 °C. Protect from heat, light and moisture. Allow tablets to reach room temperature before use. Solutions should be freshly prepared.

Preparation Instructions

1. Dissolve one tablet in 0.05 M phosphate-citrate buffer, pH 5.0, to the desired concentration. Typically an OPD concentration of 0.4 mg/mL is used.
2. Add 40 µL of fresh 30% hydrogen peroxide (e.g. Catalog Number H1009) per 100 mL of substrate buffer solution, immediately prior to use.

To prepare 0.05 M phosphate-citrate buffer, pH 5.0:

- Add 25.7 mL of 0.2 M dibasic sodium phosphate (e.g. Catalog Number S0876), 24.3 mL of 0.1 M citric acid (e.g. Catalog Number C7129) and 50 mL of water.
- Adjust the pH to 5.0, if necessary.

Alternatively, use phosphate-citrate buffer capsules containing sodium perborate (e.g. Catalog Number P4922). If these capsules are used, it is not necessary to add H_2O_2 to the substrate solution, since sodium perborate is a substitute for hydrogen peroxide.

Troubleshooting

<u>If the background is too high</u>:

1. Use a blocking step prior to 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:
   a. 0.05% TWEEN® 20 in 50 mM TBS, pH 8.0
   b. 1% BSA containing 0.05% TWEEN 20 in 50 mM TBS, pH 8.0
   c. 3% nonfat-dried milk in 0.01 M TBS (e.g. Catalog Number 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 non-specific 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 test 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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