

Product Information

o-Phenylenediamine dihydrochloride Tablet, 5 mg substrate per tablet

Product Number **P6912**

Storage Temperature 2–8 °C

CAS Number: 615-28-1

Synonyms: 1,2-benzenediamine, OPD

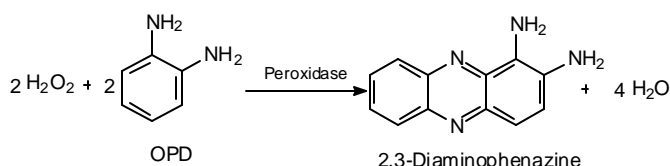
Product Description

Molecular Formula: $C_6H_4(NH_2)_2 \cdot 2HCl$

Molecular Weight: 181.06

λ_{max} : 287–291 nm

o-Phenylenediamine (dihydrochloride) is a chromogenic substrate suitable for use in ELISA procedures that utilize horseradish peroxidase conjugates.^{1,2} 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 3N HCl or 3 M H_2SO_4 solution, and read at 492 nm.



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.^{3,4}

Each tablet contains 5 mg of substrate and weighs ~150 mg. One tablet, dissolved in 10 ml of water, gives a solution with a pH of 5.0. Background absorbance (A_{450}) is not more than 0.04. This product is supplied as 50 or 100 tablets per box, individually foil wrapped for ease of use, storage, and safety.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material 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 to reach room temperature before use.

Preparation Instructions

Prepare the appropriate volume of 0.05 M phosphate-citrate buffer, pH 5.0, required for the ELISA assay.

Substrate buffer preparation options:

A. Phosphate-citrate buffer with H_2O_2

- Add 25.7 ml of 0.2 M dibasic sodium phosphate (Product No. S0876), 24.3 ml of 0.1 M citric acid (Product No. C7129) and 50 ml of deionized water. Adjust the pH to 5.0, if necessary.
- Dissolve phosphate-citrate buffer tablet, Product No. P4809, in 100 ml deionized water.

Note: Immediately prior to use, add 40 μ l of fresh 30% hydrogen peroxide (Product No. H1009) per 100 ml of 0.05 M phosphate-citrate buffer solution.

B. Phosphate-citrate buffer with sodium perborate

Dissolve the contents of a phosphate-citrate buffer with sodium perborate capsule (Product No. P4922) in 100 ml of deionized water. This yields a 0.05 M phosphate-citrate buffer containing 0.03% sodium perborate as a substitute for H_2O_2 .

Procedure

Note: For more detailed ELISA procedures, please visit the Antibody Explorer section of our website at www.sigmaaldrich.com.

1. Remove the appropriate number of OPD tablets required for the assay and return the box to the refrigerator. Allow the tablets to reach room temperature.
2. Prepare the Substrate Solution by dissolving tablet(s) 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. A 5 mg tablet dissolved in 12.5 ml of buffer provides an OPD concentration of 0.4 mg/ml. Do not touch the tablets with your fingers and do not use metallic forceps. Vortex until dissolved.

Note: If required, add hydrogen peroxide, H_2O_2 , as previously described, immediately prior to use. For best results, the solution should be used within one hour.

3. After adding the horseradish peroxidase conjugated antibody to the plate, wash thoroughly to remove unbound conjugate.
4. Add 200 µl of Substrate Solution to each well. Incubate the plate, in the dark, for 30 minutes at room temperature.
5. After the incubation period, read the plate at 450 nm on a multiwell plate reader.
6. If you can not read the plate immediately, the reaction may be stopped by the addition of 50 µl of 3 N HCl or 3 M H₂SO₄ per 200 µl of reaction solution. Read stopped reactions at 492 nm.

Troubleshooting

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 secondary antibody generally produces the best results.
2. Additional blocking agents for an ELISA are:
 - a. 0.05% TWEEN[®] 20 in 0.01M phosphate buffered saline (PBS), pH 7.4 (Product No. P3563).
 - b. PBS with 1% bovine serum albumin (BSA) (Product No. A9647) containing 0.05% TWEEN 20.
 - c. 3% nonfat-dried milk in PBS (Product 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 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 amplification system such as avidin-biotin.

References

1. Wolters, G., et al., Solid-phase enzyme-immunoassay for detection of hepatitis B surface antigen. J. Clin. Path., **29(10)**, 873–879 (1976).
2. Bovaird, J. H., et al., Optimizing the o-phenylenediamine assay for horseradish peroxidase: effects of phosphate and pH, substrate and enzyme concentrations, and stopping reagents. Clin. Chem., **28(12)**, 2423-2426 (1982).
3. Tarcha, P. J., et al., 2,3-Diaminophenazine is the product from the horseradish peroxidase-catalyzed oxidation of o-phenylenediamine. Anal. Biochem., **165(1)**, 230-233 (1987).
4. Bystryak, S. M., and Mekler, V. M., Photochemical amplification for horseradish peroxidase-mediated immunosorbent assay. Anal. Biochem., **202(2)**, 390-393 (1992).

Related Products

OPD tablet products

Product Number	Substrate per tablet	Buffer Volume*
P6662	1 mg	2.5 ml
P6787	2 mg	5 ml
P8806	3 mg	7.5 ml
P8787	4 mg	10 ml
P3804**	5 mg	12.5 ml
P6912**	5 mg	12.5 ml
P8287	10 mg	25 ml
P4664	15 mg	37.5 ml
P7288	20 mg	50 ml
P8412	30 mg	75 ml
P1063	60 mg	150 ml

*Volume of buffer required to make the typical 0.4 mg/ml substrate solution.

**Both P3804 and P6912 tablets contain 5 mg OPD substrate. However, the tablet weight of P3804 is ~16 mg, whereas, the tablet weight of P6912 is ~150 mg.

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CMH,BG,KTA 09/05-1

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