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Product Information

_o_-Phenylenediamine dihydrochloride tablet
10 mg substrate per tablet

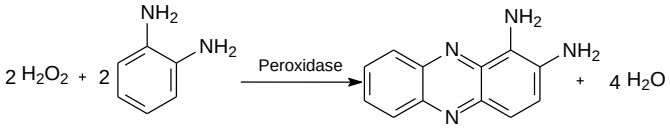
Catalog Number **P8287**
Storage Temperature 2–8 °C

CAS RN 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 3 M HCl or 3 M H_2SO_4 solution, 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.[3,4]

Each tablet contains 10 mg of substrate and weighs ~32 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 0.05. 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 Research Use Only. Not for Use in Diagnostic Procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

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 (e.g. Catalog Number S0876), 24.3 mL of 0.1 M citric acid (e.g. Catalog Number C7129) and 50 mL of deionized water. Adjust the pH to 5.0, if necessary.
  Or
- Dissolve a phosphate-citrate buffer tablet (e.g. Catalog Number P4809) in 100 mL deionized water.

Note: Immediately prior to use, add 40 µL of fresh 30% hydrogen peroxide (e.g. Catalog Number 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 (e.g. Catalog Number 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.

Storage/Stability

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

Procedure

Note: For more detailed ELISA procedures, please visit the Antibody Explorer at our website (www.sigmaaldrich.com/antibodyexplorer).

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 10 mg tablet dissolved in 25 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 cannot read the plate immediately, the reaction may be stopped by the addition of 50 µL of 3 M HCl or 3 M H_2SO_4 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 0.01 M phosphate buffered saline (PBS), pH 7.4 (e.g. Catalog Number P3563).
   b. PBS with 1% bovine serum albumin (BSA) (e.g. Catalog Number A9647) containing 0.05% TWEEN 20.
   c. 3% nonfat-dried milk in PBS (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 amplification system such as avidin-biotin.

Related Products

OPD tablet products

Catalog 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.

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*-phenylene-diamine 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).

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