Product Information

o-Phenylenediamine dihydrochloride tablet

Tablet, 5 mg substrate per tablet

P6912

Product Description

CAS Registry Number: 615-28-1

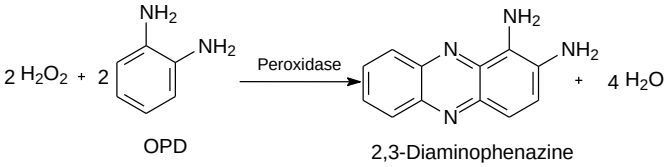
Synonyms: 1,2-benzenediamine, OPD

Molecular Formula: $C_6H_8N_2 \bullet 2\ HCl$

Molecular Weight: 181.06

λ_{max}: 287-291 nm

o-Phenylenediamine (dihydrochloride) is a chromogenic substrate that is suitable for use in ELISA procedures that utilize horseradish peroxidase (HRP) 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 OPD oxidation product that HRP produces is 2,3-diaminophenazine. 2,3-diaminophenazine has been characterized by melting point, mass spectrometry, and NMR.[3,4]

Several dissertations[5-18] have cited use of product P6912 in their research protocols.

Reagent

Each P6912 tablet contains 5 mg of substrate and weighs ~150 mg. One P6912 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.

P6912 is supplied as 50 tablets (50TAB) or 100 tablets (100TAB) per box, individually foil wrapped for ease of use, storage, and safety.

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.

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 include:

1. Phosphate-citrate buffer with H_2O_2:
   - Add 25.7 mL of 0.2 M dibasic sodium phosphate, 24.3 mL of 0.1 M citric acid and 50 mL of deionized water.
   - Adjust the pH to 5.0, if necessary.

   Or:

   - Dissolve a phosphate-citrate buffer tablet (such as Cat. No. P4809) in 100 mL deionized water.

   Note: Immediately prior to use, add 40 µL of fresh 30% hydrogen peroxide (H_2O_2) per 100 mL of 0.05 M phosphate-citrate buffer solution.
2. Phosphate-citrate buffer with sodium perborate:
   - Dissolve the contents of a phosphate-citrate buffer with sodium perborate capsule (such as Cat. 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 at our website (www.sigmaaldrich.com/antibodyexplorer).

1. Remove the appropriate number of OPD tablets required for the assay. Return the box to the refrigerator. Allow the tablets to reach room temperature.
2. Prepare the Substrate Solution by dissolving the 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.
   - Do not use metallic forceps.
   - Vortex until dissolved.
   - If required, add 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 HRP-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 the stopped reactions at 492 nm.

Troubleshooting

If 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:
   - 0.05% TWEEN® 20 in 0.01 M phosphate buffered saline (PBS), pH 7.4 (such as Cat. No. P3563)
   - PBS with 1% bovine serum albumin (BSA) containing 0.05% TWEEN® 20
   - 3% nonfat-dried milk in PBS (such as 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 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 OPD Tablet Products

Cat. No.	Substrate (mg) 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

(*) Required to make a 0.4 mg/mL substrate solution.

(**) P3804 and P6912 each contain 5 mg OPD substrate. However, their tablet weights differ:

- Tablet weight of P3804: ~16 mg
- Tablet weight of P6912: ~150 mg

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