Product Information

o-Phenylenediamine dihydrochloride

Peroxidase substrate

P1526

Product Description

CAS Registry Number: 615-28-1

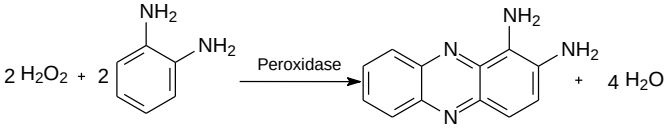
Molecular Formula: $C_6H_8N_2$ • 2 HCl

Molecular Weight: 181.06

λ_{max}: 233 nm, 289 nm (0.1 M Tris, pH 9.0)[1]

Synonyms: 1,2-benzenediamine, OPD

o-Phenylenediamine dihydrochloride is a chromogenic substrate that is suitable for use in ELISA procedures that utilize horseradish peroxidase (HRP) conjugates.[3,4] 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.[5,6]

Several theses[7-10] and dissertations[11-15] have cited use of product P1526 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.

Storage/Stability

Store the product at –20 °C. Protect from heat, light, and moisture. Allow to reach room temperature before use.

Procedure

1. Dissolve OPD 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. Immediately prior to use, add 40 µL of fresh 30% hydrogen peroxide (H_2O_2) per 100 mL of phosphate-citrate buffer solution.

Phosphate-citrate buffer

To prepare phosphate-citrate buffer, pH 5.0, mix the following:

- 25.7 mL of 0.2 M dibasic sodium phosphate
- 24.3 mL of 0.1 M citric acid
- 50 mL of deionized water

Adjust the pH to 5.0, if necessary.

Altenatively, capsules of phosphate-citrate buffer with sodium perborate (Cat. No. P4922) may be used. One P4922 capsule is added to 100 mL of deionized water. This yields a 0.05 M phosphate-citrate buffer, with 0.03% sodium perborate as a substitute for H_2O_2, so that addition of H_2O_2 is not necessary.

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 50 mM TBS, pH 8.0
   - 1% BSA (bovine serum albumin) containing 0.05% TWEEN® 20 in 50 mM TBS, pH 8.0
   - 3% nonfat-dried milk in 0.01 M 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.

References

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