Product Information

o-Phenylenediamine

Tablet, 20 mg substrate per tablet

P5412

Product Description

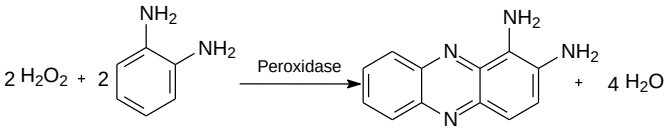
CAS Registry Number: 95-54-5
(*o*-Phenylenediamine component)

Synonyms: 1,2-benzenediamine,[1] OPD
(*o*-Phenylenediamine component)

Molecular Formula: $C_6H_8N_2$
(*o*-Phenylenediamine component)

Molecular Weight: 108.14
(*o*-Phenylenediamine component)

o-Phenylenediamine (OPD) is a chromogenic substrate that is suitable for use in ELISA procedures that utilize horseradish peroxidase (HRP) 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 solution, and read at 492 nm.



OPD 2,3-Diaminophenazine

The OPD oxidation product that HRP produces is 2,3-diaminophenazine, which has been characterized by melting point, mass spectrometry, and NMR.[4,5]

Several publications,[6-12] theses,[13,14] and dissertations[15,16] have cited use of product P5412 in their research protocols.

Reagent

P5412 is supplied as 50 tablets (50TAB) or 100 tablets (100TAB) 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 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 (H_2O_2, such as Cat. No. H1009) per 100 mL of substrate buffer solution, immediately prior to use.

Note on buffer: Phosphate-citrate buffer capsules containing sodium perborate (such as Cat. No. P4922) may be used. With these capsules, adding H_2O_2 to the substrate solution is not necessary, since sodium perborate is a substitute for hydrogen peroxide.

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.

References

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