Product Information

3,3’-Diaminobenzidine (DAB) Enhanced Liquid Substrate System

Tetrahydrochloride, for Membrane Applications

D6815

Product Description

Diaminobenzidine (DAB) is used in many applications to visualize peroxidase activity.[1-6] The DAB Enhanced Liquid Substrate System is for use in membrane ELISA procedures as a precipitating substrate for the detection of peroxidase activity. DAB is the immunohistology substrate of choice, because it produces an intense brown stain that is easily observed. The end product is resistant to alcohol. Thus, various counterstains and mounting media in alcoholic solutions can be used with the DAB Liquid Substrate System.

The DAB Enhanced Liquid Substrate System is supplied as two reagents, the buffer and the chromogen, which are mixed together immediately prior to use. The DAB Liquid Substrate System provides all the chromogen and buffer/peroxide solutions that are needed to produce a fast and convenient DAB substrate solution.

Several publications[7-10] and dissertations[11-13] have cited use of product D6815 in their research protocols.

Storage/Stability

The DAB buffer and DAB chromogen solutions are stable for up to 18 months when stored at 2-8 °C in the original containers.

After combining the two components, the resulting reagent is stable for:

- 2 to 4 hours at room temperature
- up to 8 hours at 2-8 °C

During these time periods, the 1× reagent may develop a darker color. However, the reagent performance will not be affected.

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.

Components

This DAB Liquid Substrate System product consists of the following reagents:

- DAB Liquid Buffer Solution A (Component Number D5940): 4 × 100 mL
- DAB Liquid Chromogen Solution B (Component Number D5815): 15 mL

Reagents and Equipment Required but Not Provided

- Test tubes

Procedure

1. Add one drop of the DAB Liquid Chromogen Solution B (approximately 30 µL) to a test tube. Dilute with 1 mL of DAB Liquid Buffer Solution A. Mix well. Larger quantities can be made using the same ratio of concentrate to buffer.
2. Apply a sufficient volume of the prepared DAB reagent solution to cover the membrane completely.
3. Incubate for 5 to 30 minutes. It may be useful to monitor the development of the reaction product to prevent overstaining.
4. Stop the reaction by gently rinsing the membrane in 2 to 3 changes of distilled water.
5. Allow the membrane to dry for appropriate results.

6. Dilution of the DAB Liquid reagents is **not recommended**. To reduce the intensity of a reaction, it is recommended that the antibodies or conjugates be diluted, or the reaction time be reduced.

Troubleshooting

Background too high

1. Decrease the staining time.
2. Titer the conjugate to optimize working dilution.

No color develops or 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.
4. Consider using an amplifying system such as avidin-biotin.
5. Increase the staining time.

References

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