Product Information

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

For immunohistology

D3939

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 immunohistology procedures as a precipitating substrate to detect 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 theses[7-9] and dissertations[10-28] have cited use of product D3939 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

The DAB Liquid Substrate System consists of the following reagents:

- DAB Liquid Buffer Solution A (Component Number D6190): 100 mL
- DAB Liquid Chromogen Solution B (Component Number D6065): 3 mL (packaged in a dropper bottle)

Reagents and Equipment Required but Not Provided

- 0.2 µm filter
- Tris buffered saline (TBS, such as Cat. No. T5030) for washing

Procedure

1. Add 30 µL (one drop) of the DAB Liquid Chromogen Solution to 1 mL of the DAB Liquid Buffer Solution. Mix well. For best results, use the prepared DAB reagent solution immediately.
2. Cover tissue sections with 0.2-0.5 mL of the DAB reagent solution.
3. DAB is a fast-acting substrate. Monitor carefully during the reaction to prevent over-development and high background. The reaction may be stopped by gently washing the slide in water or TBS.

4. Occasionally DAB solutions may be hazy. The haziness may be removed by filtering the DAB solution through a 0.2 µm filter.
5. When finished, dispose of any remaining substrate solution in a manner consistent with proper hazardous material handling protocols for your institution.

Troubleshooting

Background too high

1. Prior to the application of the primary antibody, block the tissue with 10% (v/v) normal serum from the host species of the secondary antibody.
2. Prior to antibody incubations, block endogenous peroxidase by flooding the slides with a solution of 4 parts of methanol to 1 part of 3% H_2O_2 solution.
3. Decrease the staining time.
4. 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.
6. Determine if enzymatic treatment (unmasking) of the antigen is required prior to application of the primary antibody.

References

1. Nakane, P.K., and Pierce, G.B., Jr., *J. Histochem. Cytochem.*, **14(12)**, 929-931 (1966).
2. Trojanowski, J.Q. *et al*., *J. Histochem. Cytochem.*, **31,** 1217-1223 (1983).
3. DeJong, A.S.H. *et al*., *Histochem. J.*, **17(10)**, 1119-1130 (1985).
4. Chu, N.M. *et al*., *J. Histochem. Cytochem*., **37(2)**, 257-263 (1989).
5. Merchenthaler, I. *et al*., "Silver Intensification in Immunocytochemistry", in *Techniques in Immunocytochemistry* (Bullock, G., and Petrusz, P., eds.). Academic Press Ltd. (San Diego, CA), pp. 217-252 (1989).
6. Hsu, S., and Soban, E., *J. Histochem. Cytochem*., **30(10)**, 1079-1082 (1982).
7. Shewale, Swapnil Vijay, "Low dose nerve agent sarin causes dilated cardiomyopathy and autonomic imbalance in mice". Wright State University, M.S. thesis, pp. 26 (2011).
8. Kim, Jin Young, "Dual functions of Yes-associated protein (Yap) in retina and retinal pigment epithelium (RPE) in eye development". Temple University, M.S. thesis, p. 28 (2014).
9. Haddad, Majd, "Roles of Yap in the metaplastic response and regeneration of the gastric epithelium". McGill University, M.Sc. thesis, p. 19 (2020).
10. Yang, Yanfei, "The study and manipulation of piglet gonocytes". University of Saskatchawan, Ph.D. dissertation, p. 99 (2010).
11. Savadi Shiraz, Elham, "Proteomics in seminal plasma as predictor of successful sperm retrieval in infertile azoospermic men". Justus Liebig University Giessen, Ph.D. dissertation, p. 36 (2011).
12. Fu, Xuebin, "Molecular mechanisms underlying GRK2 inhibition by RKIP *in vitro* and *in vivo*". ETH Zürich, Dr. sc. dissertation, p. 99 (2013).
13. Vilfranc, Chrystelle L., "Elucidating the role of BRUCE in liver disease pathogenesis". University of Cincinnati, Ph.D. dissertation, p. 74 (2013).
14. Hoang, Thi My Linh, "Engineering salinity tolerance in rice by exogenous expression of cell death regulators". Queensland University of Technology, Ph.D. dissertation, p. 110 (2014).
15. Lin, Jeremy David, "Adaptation of a Periodontally Diseased Bone-Periodontal Ligament-Tooth Fibrous Joint to Occlusal Loads". University of California San Francisco, Ph.D. dissertation, p. 82 (2015).
16. Lobato, Irene Navarro, "$RGS14_{414}$-mediated prevention of an episodic memory loss: a study of molecular mechanism". Universidad de Malaga, Ph.D. dissertation, p. 49 (2015).
17. Poloni, Alana, "Investigation of host specificity mechanisms of *Sporisorium reilianum* in maize and sorghum". RWTH Aachen University, Dr. rer. nat. dissertation, p. 22 (2015).

18. Szabó, Réka, "Regeneration and Calcification in the Spirobranchus Lamarcki Operculum: Development and Comparative Genetics of a Novel Appendage". University of St. Andrews, Ph.D. dissertation, p. 18 (2015).
19. Akladios, Bassem, "The Role of Yes-Associated Protein (YAP) in Skin Regeneration". University of New South Wales, Ph.D. dissertation, p. 68 (2017).
20. Kar, Swayamsiddha, "Investigation of the Epigenetic Regulators and Signaling Pathways influencing Pluripotency Inducing Transcription Factors mediated Tumorigenesis". National Institute of Technology Rourkela, Ph.D. dissertation, pp. 39, 72, 94 (2017).
21. Sheftel, Yevgeny, "Novel grafting materials for sinus floor elevation in the sheep model". University of Otago, D. Clin. Dent. dissertation, p. 137 (2017).
22. Wolf, Stefan, "Requirements for cardioprotective GRK2 inhibition *in vitro* and *in vivo*". ETH Zürich, Dr. sc. dissertation, p. 35 (2018).
23. Yin, Xin, "Pharmacological Modulation of Mood, Behaviour and Cognition". University of Tasmania, Hobart, Ph.D. dissertation, p. 85 (2018).
24. Jamous, Yahya Fahad M., "Identification of *SCD1* as a heart failure-promoting gene by whole genome microarray gene expression profiling and transgenic techniques". ETH Zürich, Dr. Sc. dissertation, p. 48 (2019).
25. Dimri, Shalini, "Molecular Imaging of STAT3 Signaling *In Vivo*". Homi Bhabha National Institute, Ph.D. dissertation, p. 196 (2020).
26. Giles, Nichole, "Investigating mechanisms downstream of (Yes-Associated Protein) YAP involved in skin regeneration using artificial human skin models". University of New South Wales, Ph.D. dissertation, p. 34 (2020).
27. Kaiser, Helen Elizabeth, "The role of kynurenine, a tryptophan metabolite that increases with age, in muscle atrophy and lipid peroxidation". Augusta University, Ph.D. dissertation, p. 31 (2020).
28. Ullah, Rahim, "Deciphering the molecular basis of trophoblast stem cell differentiation and function". Lahore University of Management Sciences, Ph.D. dissertation, p. 34 (2020).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

D3939dat Rev 03/22 FEB,GCY,MAM