

## Product Information

**SIGMAFAST™ 3,3'-Diaminobenzidine tablets**

Tablet, to prepare 5 mL

**D4293****Product Description**

Diaminobenzidine (DAB) is used in many applications for visualization of peroxidase activity.<sup>1-6</sup> DAB is the immunohistology substrate of choice, as it produces an intense brown-black stain, which is resistant to alcohol. Slides stained with DAB may be cover-slipped in the traditional manner and stored for future reference.

SIGMAFAST™ 3,3'-Diaminobenzidine tablets have been developed for use in immunohistology as a precipitating substrate for the detection of peroxidase activity:

$$\text{Peroxidase} + 2 \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2 \text{H}_2\text{O} \text{ (pH 7.6)}$$
$$\text{O}_2 + \text{DAB} \rightarrow \text{insoluble, brown-black precipitate}$$

SIGMAFAST™ DAB tablets require no additional ingredients or procedures to prepare an active substrate solution. One SIGMAFAST™ DAB tablet set (one DAB tablet and one Urea Hydrogen Peroxide tablet) dissolved in 5 mL of ultrapure water provides 5 mL of a ready-to-use substrate solution that contains:

- 3,3'-Diaminobenzidine (DAB): 0.7 mg/mL
- Urea Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub> equivalence, 0.7 mg/mL): 2.0 mg/mL
- Trizma® buffer: 60 mM

This product has been used in studies on such systems as hepatocellular carcinoma,<sup>7</sup> gastric carcinoma,<sup>8</sup> *Xenopus laevis* muscle and embryos,<sup>9</sup> mouse models,<sup>10-14</sup> *Zootoca vivipara* embryos,<sup>15</sup> dogfish testes,<sup>16</sup> rainbow trout,<sup>17</sup> Sprague-Dawley rats,<sup>13</sup> and food samples.<sup>18</sup> Several theses<sup>19-22</sup> and dissertations<sup>23-36</sup> have cited use of product D4293 in their research protocols.

**Storage/Stability**

Store the tablets at -20 °C.

**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**

- 3,3'-Diaminobenzidine (DAB) tablets (Component Number D9292): 5SET (5 tablets) or 50SET (50 tablets)
- Urea Hydrogen Peroxide tablets (Component Number U1380): 5SET (5 tablets) or 50SET (50 tablets)

**Reagents and Equipment Required but Not Provided**

- Ultrapure water (17 MΩ•cm or equivalent)
- Pipette capable of delivering 5 mL
- Test tubes

**Additional Optional Materials**

- 0.2 µm filter (such as Cat. No. WHA10462701)
- Nickel(II) chloride (NiCl<sub>2</sub>) or Cobalt(II) chloride (CoCl<sub>2</sub>), to prepare 0.3% (w/v) stock solution to enhance tissue stains
- PBS for washing

**Preparation Instructions**

1. Remove the required number of DAB and Urea Hydrogen Peroxide tablets from the freezer.
2. Allow the tablets to reach room temperature.
3. Open DAB tablet package (silver foil) and Urea Hydrogen Peroxide tablet package (gold foil). Drop the tablets into an appropriate container. **Do not touch the tablets with your fingers.**

4. Add 5 mL of ultrapure water.
5. Vortex until dissolved.

The SIGMAFAST™ DAB Substrate Solution is now ready for use. For best results, the solution should be used within one hour.

## Procedure

1. Cover the treated tissue section with 0.2-0.5 mL of DAB Substrate Solution.
2. The DAB reaction may occur rapidly. Color development should be carefully monitored during the reaction to prevent overdevelopment and high backgrounds. Reactions may be stopped by gently washing the slide in water or PBS.
3. DAB reactions may be enhanced by the addition of a NiCl<sub>2</sub> or CoCl<sub>2</sub> solution. Add 0.5 mL of 0.3% (w/v) stock solution to 4.5 mL DAB Substrate Solution. The addition of metal salts to DAB changes the color of the precipitate product to black or blue-black.
4. Occasionally the DAB Substrate Solution may be hazy. The haziness may be removed by filtering the solution through a 0.2 µm filter.
5. Tissues stained with SIGMAFAST™ DAB Substrate Solution may be dehydrated with alcohol and mounted with traditional resinous mounting media.

## Troubleshooting

### Background is too high

1. Use a blocking step prior to the application of the primary antibody. Diluted normal serum (10% v/v) from the same species as the secondary antibody generally produces the best results.
2. Block endogenous peroxidase by flooding the slide with a solution of 4 parts methanol and 1 part 3% H<sub>2</sub>O<sub>2</sub> 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 or peroxidase anti-peroxidase.
5. Increase the staining time.

6. Determine if enzymatic treatment (unmasking) of the antigen is required prior to application of the primary antibody.

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