

Diaminobenzidine

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Diaminobenzidine

1 INTRODUCTION/SCOPE

Diaminobenzidine is used by FBI Laboratory Friction Ridge Discipline personnel to develop latent prints and enhance visible prints deposited in blood on porous, semi-porous, and non-porous items (most effective on porous items).

2 STANDARDS AND CONTROLS

See *Processing Overview* (FRD-300).

3 LIMITATIONS

None

4 EQUIPMENT

- Distilled water
- 3,3'-Diaminobenzidine Tetrahydrochloride (purity \geq 97%)
- Hydrogen Peroxide (30% Solution)
- 1 M Phosphate Buffer Solution (pH 7.4)
- 5-Sulfosalicylic Acid (purity \geq 99%)

5 PROCEDURE

5.1 Solution Preparation

Personnel will prepare the solutions as follows: [Alternative amounts of the working solution may be prepared, provided the same ratio of chemicals mixed is retained.]

5.1.1 Solution A (Fixer solution)

- A. Combine:
 - 5-Sulfosalicylic Acid - 20 g
 - Distilled water - 1000 mL
- B. Stir until 5-Sulfosalicylic Acid dissolves.

5.1.2 Solution B (Buffer solution)

- A. Combine:
 - 1 M Phosphate Buffer Solution - 100 mL
 - Distilled water - 800 mL
- B. Mix thoroughly.

5.1.3 Solution C (Diaminobenzidine solution)

- A. Combine:
 - 3,3'-Diaminobenzidine Tetrahydrochloride - 1 g
 - Distilled water - 100 mL
- B. Stir until 3,3'-Diaminobenzidine Tetrahydrochloride dissolves.

5.1.4 Working solution

- A. Combine:
 - o Solution B (Buffer solution) - 180 mL
 - o Solution C (Diaminobenzidine solution) - 20 mL
 - o Hydrogen Peroxide (30% solution) - 1 mL
- B. Mix thoroughly.

5.2 Application

Personnel will complete the following steps in order:

- A. Apply Solution A (Fixer solution) to the item.
 - 1. Leave solution on the item for 3 to 5 minutes.
- B. Apply distilled water to item.
 - 1. Leave water on the item for 30 to 60 seconds.
- C. Apply working solution to the item.
 - 1. Leave solution on the item for up to 5 minutes.
- D. Apply distilled water to the item to stop development.
 - 1. Allow item to dry.
- E. Solutions may be reapplied as needed using steps A through D.
 - 1. Personnel will be cautious of overdevelopment and destruction of background.
- F. Preserve appropriate friction ridge detail as applicable (e.g., digitally).

5.3 Storage

Solution A (Fixer solution) and Solution B (Buffer solution) must be stored in dark bottles.

5.4 Shelf Life

- A. Solution A (Fixer solution) and Solution B (Buffer solution) have indefinite shelf lives provided the reagent checks are satisfactory.
- B. Solution C and working solution are not retained and are prepared as needed.

6 SAFETY

See FBI Laboratory Safety Manual for appropriate information.

7 REVISION HISTORY

Revision	Issued	Changes
03	08/17/2022	Reformatted Section 5.2 – Added description of tissue method
04	10/15/2024	Section 1 – Scope wording modified. Section 5.1.2-B – added. Section 5.2 – application streamlined. Section 5.3 and Section 5.4 – Solution C no longer stored.