Diaminobenzidine

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Diaminobenzidine

1 INTRODUCTION/SCOPE

A. Diaminobenzidine is used by FBI Laboratory Friction Ridge Discipline personnel to develop latent prints and enhance visible prints deposited in blood.
B. The process can be used on all surfaces but is primarily used on porous items.

2 STANDARDS AND CONTROLS

See *Processing Overview* (FRD-300).

3 LIMITATIONS

None

4 EQUIPMENT

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

5 PROCEDURE

5.1 Solution Preparation

Personnel will prepare the solutions as follows. Alternative amounts of the final 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

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 Developer solution

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

B. Mix thoroughly.

5.2 Application

A. Personnel will complete the following steps in order:
   1. Apply Solution A (Fixer solution) to item by spraying, submersion, squirting, or painting.
      i. Application can also be accomplished by the tissue method which involves wetting a durable tissue material and applying the material directly to the surface or by applying through a durable tissue material onto the surface.
      ii. Leave on item for 3 to 5 minutes.
   2. Apply distilled water to item by spraying, submersion, squirting, painting, or tissue method.
      i. Leave on item for 30 to 60 seconds.
   3. Apply Developer solution to item by spraying, submersion, squirting, painting, or tissue method.
      i. Leave on item to achieve maximum contrast and development but do not exceed 5 minutes.
   4. Apply distilled water to item by spraying, submersion, squirting, painting, or tissue method to stop development process.
   5. Allow item to dry.

B. Capture appropriate friction ridge details as applicable (digitally or photographically).

5.3 Storage

A. Solution A (Fixer solution) and Solution B (Buffer solution) may be stored in any type of laboratory accepted receptacle.
B. Solution C (Diaminobenzidine solution) must be stored frozen in a container that can withstand extreme cold.
C. Storage is not applicable to developer solution. It is prepared as needed.

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 (Diaminobenzidine solution) has a 6 month shelf life when frozen, provided the reagent checks are satisfactory.
C. Developer solution is not retained and is prepared as needed.

6 SAFETY

See FBI Laboratory Safety Manual for appropriate information.

7 REVISION HISTORY

Revision	Issued	Changes
02	07/15/2021	Replace Latent Print Units with Friction Ridge Discipline. Minor wording changes. Term "specimen" changed to "item" throughout. Streamline equipment list. Re-organization and re-numbering of sections. Section 1 - added last sentence. Section 3.1 - separated out into Section 3.1.1, Section 3.1.2, Section 3.1.3, and Section 3.1.4 and added ratio allowances for solutions. Section 4 - added Preamble reference.
03	08/17/2022	Reformatted Section 5.2 – Added description of tissue method