

Section F	DAB (Diaminobenzidine)	Subsection 3
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Name of Procedure:

DAB (Diaminobenzidine)

Suggested Uses:

DAB is used to develop and enhance latent impressions which have been deposited in blood. The impressions developed or enhanced with DAB will be difficult to remove and this must be considered prior to treatment. Mixing and application procedures for DAB must be followed precisely to develop bloody impressions.

Equipment Needed to Perform Procedures:

- A - Rubber apron and rubber gloves
- B - Face shield visor and/or safety goggles
- C - Magnetic stirrer, magnetic follower and magnetic retriever
- D - Two (2) glass beakers
- E - Dark glass bottles
- F - Dark shatterproof containers
- G - Glass trays
- H - Measuring cylinders
- I - Weighing scales
- J - Four (4) processing trays
- K - Tissue
- L - Spray or squirt bottle for Tissue method
- M - Camera (35 mm, 2 1/4, MP-4, CU5, TC III)

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N - Fume hoods

O - Freezer for storage

Chemicals Needed For Preparation of Chemical Solution(s):

A - Twenty (20) grams of 5-Sulfosalicylic Acid

B - One-Hundred (100) ml of 1M phosphate buffer (pH of 7.4)

C - One (1) gram of DAB (Diaminobenzidine)

D - One (1) ml of 30 % Hydrogen Peroxide

E - Distilled Water (The use of distilled or purified water is required for this process).

Formula/Directions for Preparation of Chemical Solution(s):

Stock Solutions:

Solution A - 2 % Sulfosalicylic Acid Solution (Fixer):

1. Place twenty (20) grams of 5-Sulfosalicylic Acid in a large beaker.
2. Add one (1) liter of distilled water to the glass beaker. Place the magnetic follower in the solution and stir until thoroughly dissolved.
3. Transfer solution A to a clean and properly labeled shatterproof container until needed (Solution A may be stored at room temperature).

Solution B - Buffer Solution:

1. Place one-hundred (100) ml of 1M phosphate buffer (pH of 7.4) in a large beaker.
2. Add eight-hundred (800) ml of distilled water to the buffer. Place the magnetic follower in the solution and stir until thoroughly dissolved (This solution may be mixed manually).

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3. Transfer solution B to a clean and properly labeled shatterproof container until needed (Solution B may be stored at room temperature).

Solution C - [3,3- Diaminobenzidine (DAB)]:

1. Place one (1) gram of DAB in a large beaker.
2. Add one-hundred (100) ml of distilled water to the beaker. Place the magnetic follower in the solution and stir until thoroughly dissolved.
3. Transfer solution C to a clean and properly labeled extreme cold resistant plastic bottle. Solution C must be stored in a freezer until needed.

Developer Solution:

Note: The developer solution should be mixed just prior to usage and in the following order:

1. Place one-hundred eighty (180) ml of Solution B in a large clean beaker.
2. Add twenty (20) ml of Solution C. Place the magnetic follower in the solution and stir until thoroughly dissolved.
3. Add one (1) ml of 30 % Hydrogen Peroxide and continue to stir until thoroughly dissolved.

Processing Procedures for Application to Item(s) of Evidence:

Note: All visible impressions should be photographed prior to treatment with the DAB solutions.

Dab may be applied to items of evidence in one of two (2) methods:

Submersion Method:

1. Prepare four (4) processing trays with the following solutions:

Tray #1 - Solution A (Fixer solution)

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Tray #2 - Distilled Water
Tray #3 - Developer Solution
Tray #4 - Distilled Water

2. Submerge the item in tray #1 (Solution A) for approximately three (3) to five (5) minutes.
3. Submerge the item in tray #2 (Distilled water) for approximately thirty (30) seconds to one (1) minute to rinse the item.
4. Submerge the item in tray #3 (Developer solution) for five (5) minutes. The item may be removed at any time in which adequate development has been achieved.
5. Allow the item to completely air-dry prior to proceeding.

Tissue Method:

The tissue utilized in this method should be of a quality which is durable enough to be removed from an area while wet. Do not use perfume or scented tissues; however, unscented facial and hand tissues are acceptable.

1. Prepare three (3) squirt or spray bottles with the following solutions:

Squirt Bottle #1 - Solution A (Fixer solution)
Squirt Bottle #2 - Distilled Water
Squirt Bottle #3 - Developer Solution
2. Place tissue(s) over the area to be processed.
3. Utilizing squirt bottle #1 (Fixer solution), squirt the area to be processed. The tissue will adhere to the area being processed and should remain wet and remain over the area for three (3) to five (5) minutes. If the tissue becomes dry, the solution may be reapplied.
4. Remove the tissue and squirt the area being processed with bottle #2 (Distilled Water) for approximately thirty (30) seconds to one (1) minute.
5. Apply new tissue(s) to the area being processed. Utilizing squirt bottle #3

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(Developer Solution), squirt the area to be processed. The tissue will adhere to the area being processed and should remain wet and over the area for (5) minutes. If the tissue becomes dry, the solution may be applied again. The tissue may be removed at any time in which adequate contrast is achieved or to avoid over development.

6. Remove the tissue and squirt the area being processed with bottle #2 (Distilled Water) for approximately thirty (30) second to one (1) minute.
7. Allow the item to completely air-dry prior to proceeding.

Steps to Preserve Developed Impressions:

The most appropriate methods to preserve developed impressions is through photography, using the proper techniques (See Photographic Equipment/Procedures) and/or electronically recording the impressions (See Image Processing). The contrast of the impression(s) may be enhanced by Laser and/or Alternate Light Source examinations which may be especially useful when impressions are found on dark surfaces (See Laser/Alternate Light Sources).

Safety Concerns:

DAB should only be used in a well ventilated area such as a fume hood.

Eye protection should be worn at all times when mixing, transporting and processing items of evidence or at crime scenes. Rubber aprons and gloves should be worn at all times when handling the solutions.

This technique may be used at crime scenes if proper safety considerations are applied; however, only use in a well vented area or utilize a fan to remove the fumes produced. Crime scene processing will require the use of a dust or mist respirator.

Storage and Location of Chemicals and Solutions:

DAB, 5-Sulfosalicylic Acid and 1M phosphate Buffer solutions should be stored in the original shipping container until needed.

Hydrogen Peroxide 30 % must be stored in a refrigerator.

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Solutions A and B may be stored in separate dark bottles or containers at room temperature.

Solution C should be stored in an extreme cold resistant plastic bottle within a freezer area.

The developer solution should only be mixed as needed.

Shelf Life:

DAB, 5-Sulfosalicylic Acid and 1M phosphate Buffer solutions - Indefinite

Solutions A and B - Indefinite

Solution C when mixed may be stored in an appropriate freezer for six (6) months.

Developer solution - one (1) day if unrefrigerated and two (2) day if refrigerated.

Other Information:

All serological examinations must be conducted and collection of samples removed prior to utilizing DAB.

The item of evidence should not be exposed to super glue fumes if the DAB process will be used.
The super glue fumes will adversely effect this process.

The application of DAB will have no effect on subsequent Amido Black or Coomassie Blue examinations.

Ninhydrin used prior to DAB will not affect this process.

Use only distilled or purified water to achieve maximum effectiveness.

DAB is approximately 90 % blood specific.