



## Skin Punch Biopsy for Assessment of Epidermal Nerve Fiber Density

### Sample fixation and preparation:

Proper fixation, rinsing, and cryoprotectant are essential for optimal PGP 9.5 reaction and interpretation. **Kits have a shelf life of 3 months—specimen received in expired kits will not be processed.**

A kit contains 2 sets of vials for 2 biopsy sites. One set is labeled “Site 1” and the other set is labeled “Site 2.” Place kit immediately in a 4°C refrigerator until ready to use. **Do not use** other fixatives or buffers.

### Perform the following for each biopsy:

- Carefully place specimen in the correct site vials, submerging the tissue in the liquid for each step. Put lid on vials, making sure specimen is not caught on threads. Invert vials gently so that specimen sinks to the bottom of the vial.
  - Record on vials the location of Site 1 and Site 2 (dorsal foot, distal leg, mid-thigh, or lower abdomen). Also label each vial with:
    - Site
    - Patient name
    - Birth date
    - Side
1. Place 3-mm skin punch biopsy in **Vial A (Zamboni fixative)** as soon as it's removed from patient.
  2. Fix specimen in Vial A (Zamboni fixative) for 12 to 24 hours in a 4°C refrigerator. **Do not exceed 24 hours** as this will affect the quality of the immunohistochemistry results.
  3. After 12 to 24 hours in Vial A (Zamboni fixative), transfer specimen to **Vial B (0.1 M Sorenson's Phosphate Buffer)** to rinse for 30 minutes.
  4. After 30 minute rinse, place specimen in **Vial C (Cryoprotectant)** for shipping.
  5. Place Vial C in a 4°C refrigerator until the specimen is shipped.
  6. Complete the Epidermal Nerve Fiber Density Patient Information (T702) form; specimens will not be processed if information is not completed.

### Important considerations in performing 3-mm skin punch biopsy:

1. The standard biopsy to look for a distal small fiber sensory neuropathy includes a total of two 3-mm skin punch biopsies.  
**Note:** skin biopsies must be performed on the same side of the body.  
The below sites have normative values (other sites biopsied will not have normative values)
  - Dorsal foot (2 cm proximal to the 4th metatarsal head and take the punch biopsy between the 3rd and 4th metatarsal. The punch should not be deep [1–1.5 mm], do not get tendon).
  - Distal lateral leg (measure 10 cm up from the lateral malleolus).
  - Mid-lateral thigh (estimate ½ the distance between midpoint of the upper edge of the patella to the crease of the thigh and abdomen and move transversely to the apex of the lateral thigh).
  - Lower abdomen (divide the abdomen in quadrant using the umbilicus as your reference point. The skin punch biopsy will be taken at the midpoint of the lower abdomen quadrant).
2. Cleanse the area with alcohol swabs.
3. Inject 0.5-cc lidocaine with epinephrine intradermally forming a whitish bleb. Biopsy within the raised bleb.
  - **Do not** biopsy needle track.
  - **Do not** biopsy over pen marks.
  - **Avoid** skin sites with signs of scar, tattoo, rash, trauma, or previous surgical incision.
4. Place biopsy punch perpendicular to skin surface (do not stretch skin) and gently rotate punch until it is ½ to ¾ way to base of bevel.
5. Gently lift the dermis by one edge to elevate tissue plug and cut the connecting dermis to free the tissue.
  - **Never** grasp epidermis (upper layers of skin) with forceps. Handle gently by the deeper dermis only. The fine unmyelinated epidermal fibers will be destroyed due to crush artifact by grasping the epidermis.
  - **Do Not** allow specimen to air dry on gauze. Specimen should be placed directly in Vial A (Zamboni fixative).
  - Visualize tissue—make sure epidermis is attached to dermis and not retained in biopsy punch.

**Shipping instructions: Send only if specimen is in Cryoprotectant.**

1. Ship both the “Site 1” and “Site 2” vials (Vials C) together with the completed Epidermal Nerve Fiber Density Patient Information (T702) form.
2. Ship specimens on cool packs. Do Not Freeze!
3. If the specimen is not sent via Mayo Clinic Laboratories courier service, it should be sent via an overnight express service to:  
Mayo Clinic Laboratories  
3050 Superior Drive NW  
Rochester, MN 55901

**Questions:**

For questions or assistance with specimen preparation, call the Peripheral Nerve Laboratory at 800-533-1710 or 507-284-8065.

**Kit information:**

- Kit has a shelf life of **3 months. Specimens received in an expired kit will not be processed.**
- Order kit so it is received a day or 2 before procedure, ensuring fresh solutions and adequate time in case of shipping delays (eg, delay due to weather). To order kits, call 800-533-1710 and ask for the Peripheral Nerve Lab, or call direct to 507-284-8065.
- Kit contains 6 vials (1 set for “Site 1” and 1 set for “Site 2”).
  - (2) Vial A—filled with Zamboni fixative
  - (2) Vial B—filled with 0.1M Sorrensons phosphate buffer
  - (2) Vial C—filled with cryoprotectant

