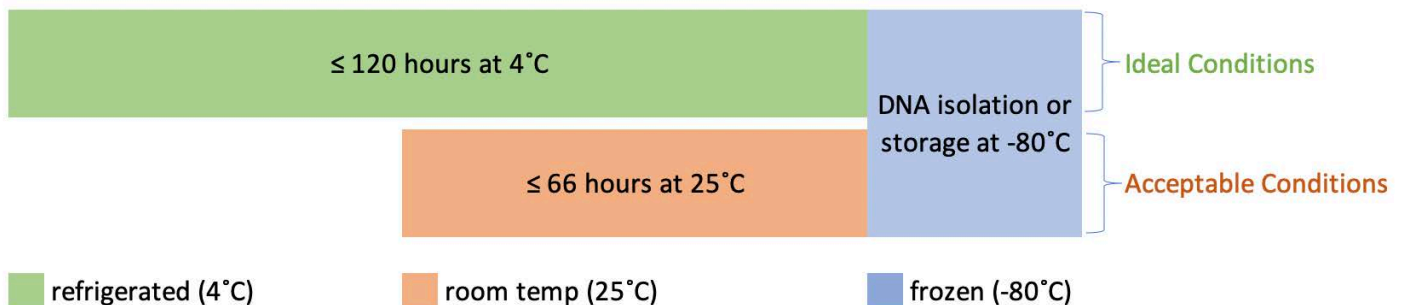


# Whole Blood Collection, Storage, and Shipping Instructions

This document is intended to give guidance as to the preferred method for collecting, storing, packaging, and shipping fresh and frozen blood to preserve DNA quality when transporting. Following these instructions will increase the likelihood that the resulting gDNA will be suitable for Bionano whole genome imaging.

## General guidelines on EDTA whole blood stability for Bionano whole genome imaging

- For best results, store EDTA whole blood at 4°C for no more than 5 days after collection, including shipping time, before DNA isolation or freezing.
  - Shipping samples with 4°C cold packs or in dry ice, following the instructions in this document, is the best way to protect samples from degradation due to extended shipping times or elevated temperatures.
- If the above scenario is not possible, samples may be maintained at room temperature for up to 66 hours, including shipping time, until DNA isolation or freezing. Intermittent exposure to elevated (30-40°C) temperatures during this time will not affect \*FSDH analysis results, but may lead to reduced DNA molecule size. Longer durations and higher elevated temperatures may affect the analysis results.



**\*Note:** Performance based on whole blood collected into EDTA tubes and validated for equivalent performance using QC metrics and FSDH calling algorithm.

## Instructions for Frozen Blood

### Materials and Equipment

The following materials are used to collect and store frozen blood:

Item	Description
Blood	≥ 650 µL (≥ 1.3 mL preferred) for samples with non-nucleated RBCs
Collection tube (EDTA preferred*)	e.g. BD Sciences, catalog # 366450
Cryovials with lid gaskets	e.g. ThermoFisher Scientific Catalog # 5011-0012
Disinfecting spray or wipes	10% bleach or equivalent
-80°C freezer	

\*NOTE: Bionano DNA Stabilizer (Bionano Genomics Catalog # 20397 or 20398) is highly recommended if Heparin collection tubes are used. DNA Stabilizer is not required when EDTA is used as an anticoagulant.

The following materials are used for shipping frozen blood to Bionano Genomics:

Item	Description
Dry Ice	≥ 5 lbs (domestic shipping) ≥ 15 lbs (international shipping)
Polystyrene box	At least 1.5 inches thick (2 inches preferred for international shipping)
Hard sided container	e.g. 50 mL conical vials or cryovial storage box
Plastic bag	e.g. Ziplock bag
Packing Slip	Provided by Bionano Genomics representative
Soft packing material	e.g. packing peanuts or bubble wrap
Customs/Shipping documents	May vary according to country of origin
Dry Ice Label	e.g. UN1845 label

## Collecting and Storing Frozen Whole Blood

Please follow these instructions when isolating blood for frozen storage and shipping. If blood has already been frozen, do not thaw to remove an aliquot. Please send the entire sample and arrange for the possibility of returning unused portion.

1. Draw blood into collection tubes. EDTA is preferred, but heparin is also accepted, following instructions below.
  - a. Adding DNA Stabilizer (Bionano Genomics, part # 20397 or 20398) to heparin blood:
    - i. For each 1 mL of blood volume in the draw tube, add 15 µl of DNA Stabilizer (Bionano Genomics, part # 20398) into the blood (e.g. add 30 ul DNA Stabilizer for 2 mL of blood volume).
    - ii. Mix blood with DNA Stabilizer thoroughly by placing the tube on a tilting rocker for 5 minutes.
    - iii. Aliquot stabilized blood by transferring each 650 µL - 1 mL volume into a 2 mL cryovial with gasket.
2. Decontaminate surface of vials using disinfecting wipes. Ensure that sample identifiers are still legible after decontamination.
3. Freeze whole blood in 650 µL - 1 mL aliquots (for non-nucleated RBCs\*) by placing aliquot into -80°C freezer as soon as possible after draw, and not more than 5 days after draw for blood in EDTA and not more than 3 days after draw for heparin blood.
  - Although only one aliquot is required for ultra-high molecular weight DNA extraction, it is recommended to freeze a second aliquot as a backup.
  - Whole blood containing nucleated RBCs may be frozen in aliquots of 100 µL.

## Packaging and Shipping Instructions for Frozen Whole Blood

Packaging materials must be leak-proof and meet the general requirements of UN3373 Category B Biological Substances as described by the [US Postal Service Packing Instruction 6F](#) (346.321) and [International Air Transportation Association Packing Instruction 650](#). Packaging should also comply with UN1845 Dry Ice requirements, as described by the [US Postal Service Packing Instruction 9A](#) and International Air Transportation Association Packing Instruction 954. Requirements of other carriers and customs authorities may apply.

1. Prepare a polystyrene box which is filled at least halfway with dry ice. Select a box that is large and thick enough to hold the samples, plus enough dry ice to keep the samples frozen during transit.
  - For domestic shipping, samples should be shipped in a polystyrene box with ≥ 5 pounds of dry ice. The walls of the box should be ≥ 1.5” thick.
  - For international shipping, samples should be shipped in a polystyrene box with ≥ 15 pounds of dry ice. The walls of the box should be ≥ 2” thick.
2. Label hard-sided secondary container(s) (e.g. 50 mL conical vials or cryotube box) and sealable plastic bag(s) “Biohazard.” Pre-chill both inside the polystyrene box with dry ice.
3. Inspect each cryovial containing frozen sample for leakage. Do not to allow sample to thaw.

4. Remove cryovials (primary container) from -80°C storage and immediately place them inside the pre-chilled hard-sided secondary container(s).
  - If flip-top microcentrifuge tubes were used instead of cryovials, seal the tubes with parafilm.
5. Securely close the secondary container(s). If using a cryotube box, use tape to prevent the lid from opening during transit.
6. Place secondary container(s) inside a sealable labeled plastic bag, along with enough absorbent material (e.g. paper towels) to absorb any liquids that may leak from the samples.
7. Immediately return the sealed bag containing packaged samples to the polystyrene box containing dry ice.
8. Cover the samples with dry ice. Any remaining empty space within the polystyrene box should be filled with additional dry ice or soft packing material.
9. Place the polystyrene box inside a final cardboard box. The polystyrene box should not be able to move inside the outer cardboard box. If necessary, add cushioning material to fill excess space.
10. Ensure that the polystyrene box and outer cardboard box are secured shut, but not airtight.
  - **Note:** The dry ice package must be able vent CO<sub>2</sub> to maintain structural integrity.
11. Apply Class 9 dry ice label (Dry Ice, UN1845) to the exterior of the box and specify the quantity of dry ice in kilograms.
12. Apply Category B Biological Substances UN3373 Diamond and add the mark “Biological Substance Category B, UN3373” to exterior of the box, along with the name and phone number of a responsible person.
13. Print a Bionano Genomics Shipping Form containing sample information. Place form in a sealed plastic bag and include inside the shipment container (affix to outside lid of polystyrene box).
14. For US domestic shipments, send the package by next-day delivery service (e.g. FedEx Priority Overnight or UPS Next Day Air). For International shipping, choose priority service.
15. Email recipient with the tracking number and the expected delivery date.
  - If shipping internationally, ensure that samples are admissible and that proper declarations are made with customs authorities. Accommodate for customs inspection accordingly. We recommend couriers that will replenish dry ice during transit and while waiting in customs, such as World Courier or FedEx International Priority with the Priority Alert Plus option selected.
  - If shipping to US Bionano Genomics headquarters, please include [shipping@bionanogenomics.com](mailto:shipping@bionanogenomics.com) in your recipient list.

Plan shipments so that they will be delivered Monday through Thursday. Shipments scheduled to arrive on Fridays are discouraged because we are unlikely to receive them over the weekend in the event of delays. Avoid shipments arriving on Saturdays, Sundays, or national holidays.

## Instructions for Fresh Blood

### Materials and Equipment

The following materials are used to collect and store fresh blood:

Item	Description
Blood	≥ 650 µL (≥ 1.3 mL preferred) for samples with non-nucleated RBCs
Collection tube	e.g. BD Sciences, Catalog # 366450

The following materials are used to shipping fresh blood to Bionano Genomics:

Item	Description
Polystyrene box	At least 1.5 inches thick
Hard sided container	e.g. 50 mL conical vials or cryovial storage box
Plastic bag	e.g. Ziplock bag
Packing Slip	Provided by Bionano Genomics representative
Soft packing material	e.g. packing peanuts or bubble wrap
Customs/Shipping documents	May vary according to country of origin
Cold packs (pre-chilled at 4°C)	e.g. Uline, Catalog # S-9906

### Collecting and Storing Fresh Blood

For best results fresh blood should be stored and shipped at 4°C, and arrive at recipient site within 5 days of blood collection, following the instructions below. If this is not possible, freeze the blood as soon as possible after collection (see Frozen Blood instructions above for preparing frozen blood for shipment). Avoid using blood that has signs of hemolysis or clotting.

1. Blood should be collected in EDTA tubes (e.g. BD Sciences, Catalog# 366450) and refrigerated as soon as possible after collection.
2. Transfer blood into cryovials with gaskets or microcentrifuge tubes sealed with parafilm. At least 650 µL blood is required for each sample.
  - a. Although only 650 µL is required for ultra-high molecular weight DNA extraction, it is recommended to prepare ≥ 1.3 mL so that the remaining volume may serve as a backup.
3. Decontaminate surface of vials using disinfecting wipes. Ensure that sample identifiers are still legible after decontamination.
4. Return blood to 4°C storage.

## Packaging and Shipping Fresh Blood

Packaging materials must be leak-proof and meet the general requirements of UN3373 Category B Biological Substances as described by the US Postal Service Packing Instruction 6F (346.321) and International Air Transportation Association Packing Instruction 650.

16. Prepare a polystyrene box which is lined with 4°C pre-chilled cold packs. Select a box that is large and thick enough to hold the samples, plus enough cold packs to keep the samples cool during transit. DO NOT use cold packs colder than 4°C, or significantly more cold packs than recommended. Incidental freeze/thaw due to too many cold packs, or cold packs that are cooler than 4°C may diminish DNA quality.
  - For domestic shipping, samples should be shipped in a polystyrene box with 4 pounds of cold packs. The walls of the box should be  $\geq 1.5$ " thick.
  - For international shipping, samples should be shipped in a polystyrene box with 6 pounds of cold packs. The walls of the box should be  $\geq 2$ " thick.
17. Label hard-sided secondary container(s) (e.g. 50 mL conical vials or cryotube box) and sealable plastic bag(s) "Biohazard." Pre-chill both inside the polystyrene box with cold packs.
18. Inspect each cryovial containing sample for leakage.
19. Remove cryovials (primary container) from 4°C storage and immediately place them inside the pre-chilled hard-sided secondary container(s).
  - If flip-top microcentrifuge tubes were used instead of cryovials, seal the tubes with parafilm.
20. Securely close the secondary container(s). If using a cryotube box, use tape to prevent the lid from opening during transit.
21. Place secondary container(s) inside a sealable labeled plastic bag, along with enough absorbent material (e.g. paper towels) to absorb any liquids that may leak from the samples.
22. Apply cushioning wrap, such as bubble wrap sheets. Wrap bubble wrap around the plastic bag until the samples are cushioned by approximately 1 inch of insulation on all sides. Secure the bubble wrap with tape. Do not use household insulation.
  - This step is critical to prevent unintended freeze/thaw, and subsequent DNA degradation.
23. Return the sealed bag containing packaged samples to the polystyrene box containing cold packs.
24. Fill any remaining empty space within the polystyrene box with additional packing material.
25. Place the polystyrene box inside an outer cardboard box. The polystyrene box should not be able to move inside the outer cardboard box. If necessary, add cushioning material to fill excess space.
26. Ensure that the polystyrene box and outer cardboard box are secured shut.
27. Apply Category B Biological Substances UN3373 Diamond and add the mark "Biological Substance Category B, UN3373" to exterior of the box, along with the name and phone number of a responsible person.

28. Print a shipping form containing sample information. Place form in a sealed plastic bag and include inside the shipment container (affix to outside lid of polystyrene box).
29. For US domestic shipments, send the package by next-day delivery service (e.g. FedEx Priority Overnight or UPS Next Day Air). For International shipping, choose priority service.
30. Email recipient with the tracking number and the shipment delivery date.
  - If shipping internationally, ensure that samples are admissible and that proper declarations are made with customs authorities. Accommodate for customs inspection accordingly. We recommend couriers that will replenish dry ice during transit and while waiting in customs, such as World Courier or FedEx International Priority with the Priority Alert Plus option selected.
  - If shipping to US Bionano Genomics headquarters, please include [shipping@bionanogenomics.com](mailto:shipping@bionanogenomics.com) in your recipient list.

Plan shipments so that they will be delivered Monday through Thursday. Shipments scheduled to arrive on Fridays are discouraged because we are unlikely to receive them over the weekend in the event of delays. Avoid shipments arriving on Saturdays, Sundays, or national holidays.

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