# Frozen Bone Marrow Aspirate Collection, Storage, and Shipping Instructions

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B

bionano

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# Revision History

| REVISION | NOTES |
|---|---|
| **A** | Initial release. |
| **B** | Updated title and content to specify Frozen BMA due to SP-G2 Fresh BMA product launch. Updated Technical Support contact numbers to include Europe. Minor grammatical corrections throughout. |

# Introduction

This document is intended to give guidance as to the preferred method for isolating, storing, packaging, and shipping **frozen bone marrow aspirates** to preserve DNA quality when transporting. Following these instructions will increase the likelihood that the resulting gDNA will be suitable for Bionano processes.

# Materials and Equipment

The following materials are used to collect and store bone marrow aspirates (**Table 1**):

**Table 1.** Collection and Storage Materials

| Item | Description |
|---|---|
| Bone Marrow Aspirates | ≥ 0.8 mL (≥ 1.6 mL preferred) |
| Collection tube (EDTA or Heparin) | e.g. BD Sciences, catalog # 366450 or 366480 |
| Bionano DNA Stabilizer | Bionano Genomics Part # 20397 or 20398 |
| Cryovials | e.g., ThermoFisher Scientific Catalog # 5011-0012 |
| Disinfecting spray or wipes | 10% bleach or equivalent |
| -80°C freezer | |

The following materials are used for shipping frozen bone marrow aspirates to Bionano Genomics (**Table 2**):

**Table 2.** Shipping Materials

| Item | Description |
|---|---|
| Dry Ice | ≥5 lbs. (domestic shipping)<br>≥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 |
| Sealable Biohazard plastic bag | General Supplier |
| Soft packing material | e.g., packing peanuts or bubble wrap |
| Absorbent material | Paper towels, Kimwipes, etc. |
| Customs/Shipping documents | May vary according to country of origin |
| Dry Ice Label | UN1845 label |

# Collecting and Storing Bone Marrow Aspirates

Please follow these instructions when collecting bone marrow aspirates (BMA) for frozen storage or shipping.

1. Draw at least 0.8 mL (1.6 mL preferred) of bone marrow into an EDTA or heparin tube.
2. Invert the BMA in the draw tube at least 10 times to fully incorporate the anticoagulant with the BMA, then keep at room temperature until aliquots are created.
3. Aliquot and freeze BMA as soon as possible after draw (but not more than 1 day after draw) following the instructions below:
    a. Mix fresh BMA thoroughly by placing the tube on a continuously mixing tilting rocker for 10 minutes.
    b. Processing one BMA sample at a time, transfer a 0.8 ml aliquot into a nuclease-free 2 mL cryovial with a gasket.
    c. Add 12 µl of DNA Stabilizer (Bionano Genomics, part number 20397 or 20398) into the 2 mL tube containing fresh BMA drawn into the Na Heparin tube.

    **NOTE:** DO NOT add DNA Stabilizer to fresh BMA drawn into EDTA tube(s).

    d. Cap tubes and invert 10 times to mix. Pulse-spin tubes for one second to collect any material from the microcentrifuge tube lids.
    e. Repeat until desired number of aliquots are created, or entire volume of BMA is transferred.

    **NOTE:** Although only one aliquot is required for UHMW DNA extraction, it is recommended to freeze a second aliquot as a backup.

    f. Decontaminate surface of vials using disinfecting wipes. Ensure that sample identifiers are still legible after decontamination.
    g. Freeze the aliquots immediately by placing them at -80°C.
    h. Store samples at -80°C.

    **WARNING:** Once frozen, the BMA may not be thawed until immediately before gDNA extraction with a Bionano-developed gDNA Isolation Protocol. If a larger aliquot has already been frozen, do not thaw and re-freeze the sample to make smaller aliquots. Users should only thaw at the time of gDNA isolation.

# Packaging and Shipping Instructions

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 that is large and thick enough to hold the samples and enough dry ice to keep the samples frozen during transit. Surround the samples with the dry ice.

- 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 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 plastic bag, along with enough absorbent material (e.g., paper towels) to absorb any liquid 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 to vent $CO_2$ to maintain structural integrity.
11. Apply a 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 a 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 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.

# Technical Assistance

For technical assistance, contact Bionano Technical Support.

You can retrieve documentation on Bionano products, SDS's, certificates of analysis, frequently asked questions, and other related documents from the Support website or by request through e-mail and telephone.

| TYPE | CONTACT |
|---|---|
| **Email** | support@bionano.com |
| **Phone** | Hours of Operation:<br>Monday through Friday, 9:00 a.m. to 5:00 p.m., PST<br>US: +1 (858) 888-7663<br><br>Monday through Friday, 9:00 a.m. to 5:00 p.m., CET<br>UK: +44 115 654 8660<br>France: +33 5 37 10 00 77<br>Belgium: +32 10 39 71 00 |
| **Website** | www.bionano.com/support |
| **Address** | Bionano, Inc.<br>9540 Towne Centre Drive, Suite 100<br>San Diego, CA 92121 |

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