# Guidelines for sample preparation and shipping for DNA/RNA extraction at Eurofins

Eurofins Genomics

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## Content

# 1. Service workflow and general guidelines for preparation and shipping

## 1.1. Sample assignment

- The sample assignment must be done in your online account.
- The sample submission form can be downloaded during the acceptance process of your online quote or order
- Assign your samples to the corresponding barcodes used in your online account
- Provide information about all quality and quantity measurements performed at your side to assure fast and optimized sample processing
- Receive UPS Labels for shipment free of charge within the European Union (no dry ice shipment)

## 1.2. Sample shipment

- Assure that all information and documents (e.g., offer confirmation in written form) are available at project start.
- Packages should be shipped overnight Monday to Thursday, especially if samples must be cooled.
- Customers outside the European Union should enclose a “proforma invoice” to declare sample value and provide general descriptions.
- Please note, that the DropBoxes / Collection Points cannot be used for the shipment of NGS samples.
- Samples must be labelled with Eurofins Barcodes. Wrong or missing labelling will result in delay of TAT and/or extra charges.
- Please specify any sample treatments performed prior to shipment (e.g. freeze-drying or enzymatic treatments) as this may alter sample quality and result in a different extraction process
- Please note that the success of the extraction process is heavily dependent on proper sampling, storage, and shipping protocols. As such, we reserve the right to process samples at the customer's own risk. Certain sample types may require a pilot setup. For more details, please contact customer support.
- Repeated freeze-thaw cycles should be avoided or kept to a minimum as they enhance nucleic acid degradation.
- For all projects a hard copy of the completed “risk group classification” (RGC) must be enclosed. You can find the risk group classification sheet: https://eurofinsgenomics.eu/media/1610544/f-1056_risk-group-classification-of-pathogenic-samples.pdf

**Samples with potential S2 organisms**:
Samples need to be packed in sealed & thick plastic bags before placing in the transport box. For more information, please consult the respective guidelines, e.g. UN3373 (“Shipping Dangerous Goods UN3373 Biological Substance, Category B).

- If you send samples with genetically modified organisms (GMOs) a separate GMO declaration form must be filled out and enclosed https://eurofinsgenomics.eu/ngs-guide.
- Please be aware: Samples with higher biosafety level than S2 are not accepted. GMOs are only accepted with biosafety level S1

**Adress**:

- Send labeled samples to

*Eurofins Genomics*
*Extraction Lab*
*Jakob-Stadler-Platz 7*
*78467 Konstanz*
*Germany*

*Please note: If you requested a quote with a high number of samples or water samples for extraction, kindly review your quote. In some cases, these samples may need to be shipped to Ebersberg for processing:*

*Eurofins Genomics Europe Food/Environment/White Biotech Products & Services GmbH*
*Extraction Lab*
*Anzinger Str. 7a*
*86650 Ebersberg*
*Germany*

### 1.3. Contact

In case of any questions, please do not hesitate to contact Customer Care by email (ngs-support@genomics.eurofinseu.com) or by phone.

All phone numbers are available at: www.eurofinsgenomics.eu/phone

Operating hours: Mondays to Fridays from 8 am to 6 pm CET.

## 2. Details for sample types

### 2.1. FFPE tissue

- To keep the rate of cross-linking and fragmentation of RNA / DNA at a minimum the tissue should be fixed as quickly as possible.
- Make sure that samples are completely dehydrated prior to embedding.
- FFPE samples must be delivered as slices (unstained and uncovered) and should be as freshly cut as possible. Delivery as block is only possible after consultation.
- Slices should not be thicker than 10 µm (surface approx. 250 $mm^2$) as the RNA / DNA yield decreases with increasing thickness.
- FFPE samples for DNA isolation can be sent at room temperature.

### 2.2. Human, animal and plant tissue

- Tissue must be snap-frozen in liquid nitrogen immediately after collection.
- To facilitate fast freezing, the tissue should be split into several pieces.
- Plant tissue max. 100 mg, other tissue max. 30 mg in 1,5 - 2 ml Eppendorf tubes
- Tissue must be shipped on dry ice.
- Samples for RNA extraction should be stored in RNAlater™ or similar stabilization reagents: 1x sample to 5x RNAlater™ is usually recommended. Please follow the manufacturer's instructions for more details.
- For HotShot crude lysate preparation, customers are requested to provide tissue samples not exceeding 1x1x1 mm, placed in 96-well plates (only available for genotyping by sequencing studies).

### 2.3. Cell culture

- Do not use lysis buffer.
- Cells must form a visible pellet, counting of cells is not required
- Snap-freeze the cells using liquid nitrogen.
- Since we perform a washing step before extraction, cells do not necessarily need to be washed. Cultured cell lines must be shipped on dry ice.
- Samples for RNA extraction must be stored in RNAlater™ or similar stabilization reagents: 1x sample to 5x RNAlater™ is usually recommended. Please follow the manufacturer's instructions for more details.

### 2.4. Blood and Plasma

- Fresh blood (whole blood) should be collected directly in appropriate tubes that already contain anticoagulant-preservative agents (like e.g. EDTA, citrate). Heparin must not be used as anticoagulant as it inhibits downstream processes such as PCR.

The maximum storage time (i.e. from sampling to nucleic acid extraction) at 4°C must not exceed 4 days.

- Blood used for RNA extraction must be collected in Paxgene RNA tubes (other tubes cannot be accepted for RNA extraction)
- For HotShot crude lysate preparation, customers are requested to provide 50µL whole blood samples, placed in 96-well plates (only available for genotyping by sequencing studies).
- Blood used for cell-free DNA extraction must be collected with Cell-Free DNA BCT® (Streck): Blood should be kept at room temperature and plasma must be prepared within 36 h. Therefore, samples must be shipped directly after collection.
- Plasma and serum samples used for cell-free DNA extraction must be snap-frozen (liquid nitrogen) immediately after collection and shipped on dry-ice.

**To separate plasma from blood samples, Eurofins recommends the following protocol:**

- Place primary blood collection tubes in a centrifuge with a swing-out rotor.
- Centrifuge the blood samples for 10 min at 1900 x g at +20 °C.
- Carefully aspirate the plasma supernatant without disturbing the buffy coat layer. About 4–5 ml of plasma can be obtained from one 10 mL primary blood collection tube.
- Transfer the aspirated plasma into fresh 15 ml centrifuge tubes with conical bottoms.
- Centrifuge plasma samples for 10 min at 16,000 x g (in fixed-angle rotor) at +4 °C.
- Carefully transfer the supernatant (plasma) into a new tube with a pipette without disturbing the pellet.
- Plasma can be kept frozen at –80 °C.

### 2.5. Bacteria and Bacteriophages

- Stab culture or agar plate can be shipped at room temperature (visible cultures must be present), glycerol stocks must be shipped on dry ice.
- Please provide us with information about the corresponding antibiotic resistances and biosafety level and enclose a hard copy of the completed “Risk group classification” (See chapter 1.2)
- Bakteriophages must not be lysed prior to shipment

### 2.6. Fermented products (cheese, yoghurt, etc.)

- Ideally, please send 5 -10 g of sample or a whole packing in a sealed container.

### 2.7. Enrichment cultures / starter cultures

- Please send a representative sample or an overnight culture freshly frozen or cooled on ice packs.

### 2.8. Swabs

- If available, please send 1 additional swab per sample.
- Please note that DNA extraction is only feasible if enough material is on the swab. This sample type will be processed on customer risk only.
- The following recommendation will improve DNA recovery:
    - **Choose the Right Swab Type**: Use swabs with synthetic fibers (such as nylon or rayon) rather than cotton, as cotton may inhibit DNA recovery. Ensure the swabs are sterile and DNA-free.
    - **Swab the Target Area Thoroughly**: Ensure you swab the surface thoroughly and evenly to maximize DNA recovery. Rotate the swab as you collect the sample, ensuring contact with all relevant areas.
    - **Use Optimal Collection Medium**: If possible, use a DNA stabilization or preservation medium (such as DNA/RNA Shield or similar solutions) immediately after swabbing to protect the DNA from degradation during transport.
    - **Avoid Contamination**: Ensure the swab is not contaminated with external sources of DNA (e.g., gloves, environment). Use proper handling techniques and sterile equipment.
    - **Store and Transport Correctly**: After collection, immediately store swabs at low temperatures (preferably frozen) to prevent DNA degradation. Transport swabs frozen on ice or in a suitable preservation medium.
    - **Minimize Drying Time**: If using dry swabs, minimize the time they are left exposed to air before freezing or placing them in a preservative. Drying can degrade DNA.

### 2.9. Human and animal feces / sewage sludge

To ensure the integrity of microbiome analyses, it is critical to follow proper sample collection, preservation, and shipping protocols. Below are the detailed instructions for sending human and animal feces or sewage sludge samples:

**Sample Quantity Requirements:**

- Please provide a **minimum of 800 mg** of frozen sample for each submission.

**Sample Preservation:**

- **Frozen Samples:**
    - Samples should be frozen immediately after collection and stored at a consistent temperature of **-80°C** until shipping.

    - Avoid freeze-thaw cycles, as repeated freezing and thawing can alter the microbiome composition, leading to compromised or inaccurate results.
- **Alternative Preservation Methods:**
    - If freezing is not feasible, you may preserve the samples using one of the following methods:
    - **Lyophilization (Freeze-Drying):**
        - Store the lyophilized feces in sterile cryotubes to prevent contamination and degradation. Ensure the tubes are properly labeled and sealed.
    - **Stabilization with Collection Systems:**
        - Utilize a stool collection and stabilization system, such as the OMNIgene®•GUT system by DNA Genotek or a similar product. These systems stabilize the microbiome for transport at ambient temperatures without compromising sample integrity.

### 2.10. Water and waste water

- Please filter 500 ml water sample through a 0.22 µm pore size filter. The filter should be filled to its full capacity to harvest $10^9$ bacterial cells. Ensure the filter is detached from the holder (i.e. not attached to a column). Before shipping, the filter should be dried and wrapped in sterile aluminium foil.
- If you do not have the possibility to filter, please contact us.

### 2.11. Sediment, Soil and Sand

- Please send a minimum of 800 mg of chilled or frozen samples, ideally in tubes.

### 2.12. Plant leaves (for surface analysis)

- Please send plant leaves in 50 ml tubes cooled or at room temperature. Depending on the size of the leaves you might want to roll the leaves. Please take extreme care to keep the leaves intact.
- For extraction of bacterial or fungal DNA we will initially detach microorganisms from the plant leaves by swirling them in a small amount of buffer to concentrate the microorganisms. Intact leaves therefore will minimize the portion of reads derived from homologous plant sequences (most relevant for 16S sequences).