

Sample Preparation and Shipping Guide

This guide provides step-by-step instructions for preparing and shipping your RNA samples to our facility for EspressoSeq gene expression analysis. Please read the entire guide, especially the material requirements below, before you begin your experiment to prevent delays.

Before You Begin: Sample & Material Requirements

RNA Sample Quality

To ensure high-quality sequencing data, each sample must contain at least 1 µg of total RNA.

- **Isolation:** While you can use any RNA isolation kit, we highly recommend using one that includes gDNA eliminator columns. An additional DNase digest is not required.
- **Quality Control:** After extraction, measure the RNA concentration using a spectrophotometer (e.g., NanoDrop). If you have concerns about RNA integrity, we suggest checking the samples on an agarose gel or with an automated electrophoresis system (e.g., Agilent TapeStation or Bioanalyzer).
- **Recommended Kits:** The following total RNA isolation kits are highly recommended as they include a compatible stabilization buffer:
 - **NEB:** Monarch Spin RNA Isolation Kit (Mini) (Catalogue #T2110S)
 - **Zymo Research:** Quick-RNA Miniprep Plus Kit (Catalogue #R1057)

Required Stabilization Buffers

To protect your samples during transit, all RNA samples must be stabilized in one of the following compatible buffers. *Note: These reagents are included in the recommended RNA isolation kits listed above.*

- **NEB:** Monarch StabiLyse DNA/RNA Buffer (Catalogue #T2111L)
- **Zymo Research:** DNA/RNA Shield, 2X Concentrate (Catalogue #R1200-25)
Important: Please ensure you purchase the 2X Concentrate version of this reagent.

The 3-Step Submission Process

Once your samples meet the requirements, follow these three simple steps.

Step 1: Register Your Project

1. Navigate to our website and fill out the **sequencing request form**. You will need to provide essential details for your project, including the species, a list of your sample names/IDs, and their corresponding experimental groups.
2. After you submit the form, we will email you a personalized **Sequencing Order PDF**.
3. To confirm your project, **print and sign this document**. You must include the signed document in the package with your samples.

Step 2: Prepare and Stabilize Samples

1. Label 1.5 ml tubes with the sample ID you entered in the form
2. Add 50 μ l of a supported stabilization buffer to each tube
3. Add 1-2 μ g of your RNA samples (max. 50 μ l) and mix by pipetting
4. Use molecular biology grade water to bring the total volume to 100 μ l
5. For extra security, seal the cap with Parafilm.

Your samples are now stable at room temperature for several days. However, if you are not shipping them immediately, we recommend storing them at 4°C or -20°C.

Step 3: Ship Your Samples

1. Place your prepared **samples** and the **signed Sequencing Order document** into a suitable shipping box.
2. During summer months (when temperatures may exceed 30°C), we recommend using an insulated Styrofoam box with approximately 2 kg of ice packs to keep the samples cool.
3. We recommend using an overnight courier (e.g., UPS, FedEx, DHL) for the fastest delivery. *Note: shipping rates can differ by more than 10 times between services.*
4. Ship the package to our sequencing facility:

XPseq Analytics GmbH
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6020 Innsbruck
Austria