Microsynth

Sample Preparation for RNA Isolation

General Information

Effective analysis depends on obtaining high-quality RNA in optimal amounts. Even within intact samples, RNA is susceptible to degradation. To mitigate this, careful handling is essential - guard against RNase contamination, use appropriate preservation buffers, and avoid freeze/thaw cycles. Adherence to the guidelines provided by Microsynth is critical: maintain the specified sample volume/weight and avoid submitting excess raw material. For added security, consider including a duplicate sample of the same quantity (cells/tissue) in a separate plastic bag for shipping.

- For >24 samples, the use of **barcoded tubes** is required; for smaller quantities, it remains an option. Please send an email to myproject@microsynth.ch with "barcodes" in the subject line and indicate the quantity of barcodes, (1 barcode / plate or tube), the quotation number of your project and the full destination address. We ship two sets of barcodes for duplicates.
- For **RNase-rich samples**, consider adding 1% beta-mercaptoethanol to lytic buffers (DNA/RNA Shield, RLT plus).
- Be sure to **follow the manufacturer's instructions** for buffer volumes; avoid overloading with excess buffer.
- For optimal stability, ship your samples at the **recommended temperatures** according to the buffer manufacturer's guidelines. We strongly recommend using dry ice for shipping to **ensure maximum sample preservation**. Try to ship your package on a Monday, Tuesday, or Wednesday to ensure that it doesn't travel over weekends.

Important:

- **Send duplicates** of your samples whenever possible. If the nucleic acid isolation does not pass the quality control, we will perform a new isolation free of charge.
- For sample types **not listed** here (e.g., tissue types such as bone or adipose tissue, very low cell numbers, special preservation buffers, plants, insects, blood, or prokaryotic pathogens in tissue), please contact your sales representative.
- We provide **additional services** at an extra cost, such as subsampling, specialized handling, method development, and more.

Eukaryotic Cell Lines

Please provide the required number of cells as indicated in Table 1 along with the appropriate buffer volume as indicated in Table 2. Do not exceed the specified number of cells, but do include duplicates. If your cell line is not listed, refer to the relevant literature to estimate the RNA yield for an accurate submission.
When registering your samples in our webshop, please specify the cell line, cell count, and preservation buffer used. Please note that incomplete information, inaccurate cell counts or buffer volumes may result in reduced RNA quality and quantity. Your attention to detail is critical for optimal results.

Table 1: *Required number of cells*

High Yield Cells	Medium Yield Cells	Low Yield Cells
COS, HUV-EC-C	HeLa, LMH, Huh, HEK 293, THP1	NIH, 3T3, PBMC, U-266, primary cells
$0.5*10^6$ to $3*10^6$ cells	$1*10^6$ to $5*10^6$ cells	$3*10^6$ to $5*10^6$ cells

Important

- Ship your samples in 2 mL barcoded snap-cap or screw-cap tubes. For more than 24 samples, submission in a 96-well deep well plate is possible.
- A 96-well plate **must be filled column wise**, otherwise extra costs will be charged.
- **Avoid submitting cells in freezing medium** (e.g. 10% DMSO), as the thawing process induces changes in the gene expression profile, and RNA quality will be low due to dead cells.

Table 2: *Preservation buffer volumes and sample shipment guide for eukaryotic cell lines*

Buffer	Sample Preservation	Storage and Shipment
ZymoResearch DNA/RNA Shield (#R1100)	Pellet cells, remove supernatant and add 300 µL for $1*10^6$ cells, 600 µL for $1*10^7$ cells	**Store:** Ambient temperature or fridge < 30 days; freezer indefinitely **Ship:** Ambient temperature, ice pads or dry ice
Qiagen RLT plus (#1053393), **do not use RLT buffer**	Pellet cells, remove supernatant and add 350 µL for < $5*10^6$ cells, 600 µL for $5*10^6$ – $1*10^7$ cells	**Store:** In fridge for < 7 days; freezer indefinitely **Ship:** Ambient temperature or ice pads within 3 days, or dry ice
Cell pellet without buffer (either snap frozen in liquid nitrogen or pre-treated with e.g. Qiagen RNAprotect Cell Reagent #76104)	Pellet cells, remove supernatant, preferably wash with PBS, and freeze in liquid nitrogen Or: mix 5 volumes of RNAprotect with 1 volume of cell culture, centrifuge for 5 min at 5k g and discard supernatant	**Store:** Ultra deep freezer **Ship:** On dry ice

Check and strictly follow the manufacturers instructions for buffer volumes and transport conditions.

Animal Tissue

Please send the specified amount of tissue as indicated in Table 3, along with the appropriate buffer volume as indicated in Table 4. While care should be taken not to exceed the specified amount of tissue, the inclusion of duplicates is encouraged. Typically, a 3 mm (equivalent to 27 mm^3) cube of most tissues weighs approximately 30 - 35 mg.
When registering your samples in our webshop, please provide details about the tissue type, organism and preservation buffer used. It is important to provide comprehensive information to avoid problems. Correct specifications and adherence to tissue weight and buffer volume are critical to the success of the RNA isolation.

Table 3: *Required amount of tissue*

High Yield Tissue	Low Yield Tissue
Spleen, Liver, Thymus	Brain, Heart, Muscle, Lung, Kidney, Embryo, Ovary
10 – 25 mg	20 – 40 mg

Your tissue is not listed? Please contact your sales representative.

Important

- Ship your samples in 2 mL barcoded snap-cap or screw-cap tubes.
- Cut tissue samples into pieces no larger than 0.5 cm in any dimension, as larger pieces will cause the preservative buffer to diffuse too slowly into the interior, resulting in RNA degradation. Be sure to completely submerge the sample in the preservation buffer.

Table 4: *Preservation buffer volumes and sample shipment guide for animal tissue*

Buffer	Sample Preservation	Storage and Shipment
ZymoResearch DNA/RNA Shield (#R1100)	30 mg of tissue in 300 µL, 60 mg in 600 µL	**Store:** Ambient temperature or fridge < 30 days; freezer indefinitely **Ship:** At ambient temperature, ice pads or dry ice
Qiagen RLT plus (#1053393), **do not use RLT buffer**	<20 mg tissue in 350 µl RLT plus, 20 – 40 mg tissue in 600 µl RLT plus	**Store:** In fridge for < 7 days; freezer indefinitely **Ship:** At ambient temperature or ice pads within 3 days, or dry ice
Invitrogen RNAlater Stabilization Solution (#R0901-100ML)	Place fresh tissue in 5–10 volumes of RNAlater solution, store in fridge overnight to allow solution to penetrate tissue, then freeze.	**Store:** Ambient temperature < 7 days, in fridge < 30 days; freezer indefinitely **Ship:** At ambient temperature, ice pads or dry ice
Qiagen RNAprotect tissue reagent (#76104)	Immerse tissue in at least 12 volumes reagent per 1 volume tissue (as a general rule, use 750 µL reagent for up to 40 mg sample)	**Store:** Ambient temperature < 7 days, in fridge < 30 days; freezer indefinitely **Ship:** At ambient temperature or ice pads within 3 days or on dry ice
Frozen without buffer	Snap freeze in liquid nitrogen	**Store:** In ultra deep freezer **Ship:** On dry ice

Check and strictly follow the manufacturers instructions for buffer volumes and transport conditions.

Cultured Bacteria and Yeast

Please provide the required number of cells as indicated in Table 5 either as a frozen pellet or along with the appropriate volume of buffer as indicated in Table 6. RNA content and quality is highly variable depending on growth conditions, media composition, treatment and species. Therefore, submission of non-listed species is possible but at your own risk. Sending duplicate samples will help optimize the RNA isolation process. Note that some bacteria and yeasts may remain viable, especially in non-lytic preservation buffers, which may affect transcription. Consult the literature prior to selection to ensure that the buffer is appropriate for your sample type.

Table 5: *Required number of cells.*

Bacteria	Yeast
Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus	*Saccharomyces cerevisiae, Pichia pastoris*
$1*10^8$ up to $2*10^9$ cells	$5*10^7$ – $2*10^8$ cells

Important

- Please include any relevant factors that downregulate transcription (e.g. rifampicin treatment) in the comments section. Failure to provide complete information, incorrect cell counts or buffer volumes may affect the quality and quantity of RNA obtained.
- To facilitate smooth processing, please use 2 mL barcoded snap or screw-cap tubes for sample submission. If sample volume exceeds 1.8 mL, consider aliquoting into multiple 2 mL tubes under the same barcode or name.

Table 6: *Preservation buffer volumes and sample shipment guide for bacteria and yeast*

Buffer	Sample Preservation	Storage and Shipment
ZymoResearch DNA/RNA Shield (#R1100)	Pellet cells, and resuspend in buffer. **Bacteria**: For 1-$5*10^8$ cells, use 350 µL. For higher numbers, for every $5*10^8$ cells, use a multiple of 350 µL. **Yeast:** For 1-$5*10^7$ yeast cells, use 600 µL. For higher numbers, for every $5*10^7$ cells, use a multiple of 600 µL	**Store:** Ambient temperature or fridge < 30 days, freezer indefinitely **Ship:** Ambient temperature, ice pads or dry ice
Invitrogen RNAlater Stabilization Solution (#R0901-100ML)	Pellet cells, add 1 mL of RNAlater for bacteria up to $2*10^9$; for yeast up to $2*10^8$, and incubate for 1 hour before freezing	**Store:** Ambient temperature < 7 days, in fridge < 30 days; freezer indefinitely **Ship:** Ambient temperature, ice pads or dry ice
Frozen without buffer	Pellet cells, preferably wash with PBS, snap freeze in liquid nitrogen **Optional** (bacteria only): To 1 volume of culture, add 2 volumes of RNAprotect bacteria reagent (#76506), mix very well, incubate 5 min at RT and centrifuge for >10 min at 5'000 g. Discard the supernatant.	**Store:** In ultra deep freezer **Ship:** On dry ice

Check and strictly follow the manufacturers instructions for buffer volumes and transport conditions.

Order Form Completion

Prior to shipping your isolation samples to Microsynth, please follow these steps to complete your order form:

1. Enter our webshop at https://srvweb.microsynth.ch/.
2. Click on „**Overview**“ under Isolation & Genetic Assaying and select the appropriate format under “**Isolation & PCR Assaying**”.
3. Fill in the order form and submit your order.
4. Prepare your samples according to this User Guide.
5. Send your samples together with the order printout and according to the listed conditions to Microsynth AG.

Shipping Address:
Microsynth AG
Isolation Department
Schützenstrasse 15
9436 Balgach
Switzerland

Need More Information?

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