

Sample Submission Instructions & Guidelines

To complete the Sample Submission Form:

NOTE: Please use the **exact shipping address** provided on page 9 of this document when shipping your samples.

1. Contact and job information
 - a. Provide the name, phone number, and email address of the primary technical contact for this project. This enables Asuragen to reach you quickly and efficiently if there are questions regarding your samples or project.
 - b. Due to varying shipment times, please enter the date when you are submitting samples.
 - c. Provide the Asuragen-assigned Job Number (or Quote Number) to ensure proper tracking of your project. Always reference this number when communicating with Asuragen about your project.
 - d. Sample storage solution information is vital to ensure proper sample processing.
2. Select the Services and Platforms which apply to your project
3. Enter your Sample Information
 - a. Enter the identifier which is affixed to each tube in your shipment package.
 - b. Enter the mass, volume, OD (A260), and tissue or cell type of the sample. These will be confirmed upon sample receipt and processing but are required in case of degradation or loss in shipment and serve as an independent confirmation of sample integrity and identity.
 - c. Factor names and levels: Provide the names of the principal factors in your experiment. These may be categorical factors, such as “cell type” or “treated/untreated” or continuous factors such as “dose” or “time”. Enter the level of the factor for each sample on the rows beneath each factor heading, (i.e. the name of the cell type or a dose as “100 mg”). These factor names and levels will be used throughout your study and conveniently reported with your tabular and graphical results.

All RNA and DNA will be assessed upon receipt: Asuragen Laboratory Operations will independently assess the amount and quality of all samples provided for mRNA, microRNA, or DNA profiling. Should there be concern about the amount or quality of a sample after this initial assessment, you will be contacted by Asuragen to decide whether to proceed, replace the sample, or drop the sample from a study.

Note: All sample input recommendations and requirements listed below include sufficient sample for upfront quality control (QC) and analysis.

Recommended Isolation Procedures

No matter the sample type, isolation method, or molecule to be analyzed, always follow these guidelines as per Asuragen's internal process:

- 1. Validate the isolation method:** Conduct a study of representative samples with your isolation method to ensure it maintains the representation, quality, and yield of the RNA or DNA required to draw valid conclusions. This often requires a carefully constructed pilot study prior to the main study.
- 2. Minimize confounding and noise:** Eliminate variances in materials, procedures, equipment, and personnel or if elimination is not feasible ensure these "noise" factors are acknowledged in your Statement of Work and built into your experimental design. Examples include using a single lot of isolation reagents and materials, standardization of all labware, and ensuring batch, operator or day-effects are characterized and minimized.
- 3. Ensure you will have sufficient samples to statistically support your conclusion (for hypothesis testing projects) or for validation (for classification projects).** The number of samples required is dependent on the variability within each experimental group inherent in the biology, collection, isolation, and analysis. This may require a pilot study to assess this variability and provide an educated estimate of the number of samples required. A minimum of three biological replicates are required for hypothesis testing.
- 4. Provide as much detail as possible on the isolation method at the time of sample submission.** If provided, this information will be reported back to you along with your experimental results so you may track isolation method performance against experimental results over time. It can also assist Asuragen Laboratory Operations in resolving anomalous results should they occur.

The following **RNA** isolation methods are generally recommended as consistent with current commercial gene expression platforms:

1. Any commercially available kits that have been shown to recover small RNAs, including miRNAs.
2. Any validated organic extraction and precipitation methods.
3. Column-based RNA isolation kits such as RNAqueous® from Ambion or RNeasy® from QIAGEN are acceptable for mRNA analysis only. These methods have not been validated by Asuragen to preserve microRNA and are therefore not generally advised.

Mass and Concentration Recommendations For All Services

Asuragen's mass and concentration recommendations below have been established to provide good quality results across a broad range of samples and diverse applications. If these cannot be met, Asuragen can process samples at the customer's written acknowledgement and acceptance of potential risks. Please contact your Business Development Manager to discuss options.

Sample Input Requirements: messengerRNA (mRNA) Analysis

For all services:

1. RNA integrity should be assessed by Agilent BioAnalyzer and have a RIN (RNA Integrity Number) of 7.0 or higher.
2. RNA purity should be assessed by spectrophotometry and have an OD260/280 ratio of between 1.6 and 2.1.
3. The total RNA submitted should be resuspended in nuclease-free water and shipped frozen on dry ice (see Shipping Instructions). Use of RNA storage solution or elution solutions (containing less than 1 mM EDTA) are acceptable and use of these solutions should be recorded in the sample submission form. Please note that the sample should be free of additives or impurities such as ethanol, guanidinium salts, EDTA (greater than 0.1 mM) or phenol.
4. Globin RNA depletion is recommended for RNA samples derived from blood (#CA0022) prior to Affymetrix or Illumina mRNA analysis, except when using NuGEN target prep methods.

Affymetrix Standard Input Service

1. Eukaryotic samples (**target prep #CA0011**):
 - a. Recommended: 1 µg RNA of concentration ≥ 200 ng/µL
 - b. Required: 250 ng RNA of concentration ≥ 20 ng/µL (12 µL for 250 ng)
2. Prokaryotic samples (**target prep #CA0013**): 1 µg RNA of concentration ≥ 50 ng/µL (20 µL for 1 µg)

Affymetrix Low-mass Input Service (target prep #CA0033):

1. 100 ng RNA ≥ 16.6 ng/µL (6 µL for 100 ng) (Eukaryotic only)

Affymetrix Service for 3' Arrays using NuGEN Ovation V2 (target prep #CA0069):

1. Recommended: 10-100 ng RNA of concentration ≥ 2 ng/µL for non-blood RNA; 20-50 ng RNA of concentration ≥ 2 ng/µL for blood RNA
2. Required: 5 ng RNA of concentration ≥ 2 ng/µL for non-blood RNA; 20 ng RNA of concentration ≥ 2 ng/µL for blood RNA

Affymetrix Service for 3' Arrays using NuGEN WT-Ovation Pico (target prep #CA0083):

1. Recommended: 1-50 ng RNA of concentration ≥ 2 ng/µL
2. Required: 500 pg RNA of concentration ≥ 2 ng/µL

Affymetrix Whole-Transcript (WT) Analysis on Gene or Exon 1.0 ST Arrays (target prep #CA0054):

1. Recommended: 1 µg RNA of concentration ≥ 300 ng/µL
2. Required: 100 ng RNA of concentration ≥ 70 ng/µL

Affymetrix WT Analysis on Gene or Exon 1.0 ST Arrays for FFPE RNA (target prep #CA0054):

1. Recommended: 1.5 µg RNA of concentration ≥ 500 ng/µL
2. Required: 1 µg RNA of concentration ≥ 300 ng/µL

Applied Biosystems TaqMan[®] Service for mRNA, individual assays (#CA0030 with technical triplicates):

1. Recommended: 50 ng per sample per assay (detector) of concentration ≥ 5 ng/µL
2. Required: 10 ng per sample per assay (detector) of concentration ≥ 5 ng/µL
 - a. Example: assessing 10 genes (assays or detectors) on one sample requires at least 100 ng input RNA and 500 ng is recommended.

Applied Biosystems TaqMan[®] Service for mRNA, array format (#CA0046 and #CA0047):

1. Recommended: 100 ng RNA per port total sample input dictated by card layout (150-850 ng per card, depending on configuration of genes); recommended concentration ≥ 10 ng/ μ L.
2. Required: 30 ng RNA per port

Sample Input Requirements: microRNA (miRNA) Analysis

For all services:

1. RNA purity should be assessed by spectrophotometry and have an OD260/280 ratio of between 1.8 and 2.1.
2. Asuragen sample QC for downstream miRNA analysis includes NanoDrop spectrophotometry and assessment of up to 3 endogenous miRNAs as a surrogate measure of overall miRNA abundance.
3. Ensure the RNA isolation method preserves the miRNA fraction (see “Recommended Isolation Procedures”) but is the TOTAL RNA fraction, not the “enriched” fraction.
4. The total RNA submitted should be resuspended in nuclease-free water and shipped frozen on dry ice (see Shipping Instructions). Use of RNA storage solution or elution solutions (containing less than 1 mM EDTA) are acceptable and use of these solutions should be recorded in the Sample Submission Form. Please note that the sample should be free of additives or impurities such as ethanol, guanidinium salts, EDTA (greater than 0.1 mM) or phenol.
5. Globin RNA depletion is not required for RNA samples derived from blood (#CA0022) prior to miRNA analysis.

Applied Biosystems TaqMan[®] Service for miRNA, individual assays (#CA0028 or #CA0029 with technical triplicates):

1. Recommended: 15 ng total RNA per assay (detector) of concentration ≥ 5 ng/ μ L
2. Required: 3 ng per assay (detector) of concentration ≥ 5 ng/ μ L

Affymetrix GeneChip[®] miRNA Array profiling service (target prep # CA0096):

1. Cell line RNA and RNA derived from FFPE samples:
 - a. Recommended: 200 ng total RNA of concentration ≥ 20 ng/ μ L (10 μ L for 200 ng)
 - b. Required: 100 ng total RNA of concentration ≥ 5 ng/ μ L (20 μ L for 100 ng)
2. RNA from tissues and whole blood:
 - a. Recommended: 100 ng total RNA of concentration ≥ 20 ng/ μ L (10 μ L for 200 ng)
 - b. Required: 75 ng total RNA of concentration ≥ 5 ng/ μ L (15 μ L for 75 ng)

Applied Biosystems TaqMan[®] Megaplex RT, Pre-amplification, and qPCR analysis using Human or Rodent miRNA Arrays Custom service offering:

1. Option for whole-genome miRNA profiling of biofluid samples yielding very limiting amounts of RNA insufficient for the methods listed above (such as plasma, serum, CSF)
2. Suggested inputs:
 - a. Recommended: ≥ 1 mL per sample for plasma, serum, or CSF
 - b. Required: ≥ 0.2 mL per sample for plasma, serum, or CSF

Sample Input Requirements: DNA Analysis

For all services:

1. DNA purity should be assessed by spectrophotometry and have an OD260/280 ratio of 1.7 to 2.1, and an OD260/230 ratio of 1.8 to 2.1.
2. DNA must be RNA-free.
3. The DNA submitted should be dissolved in nuclease-free water and shipped frozen on dry ice (see Shipping Instructions). Use of elution solutions (containing less than 0.1 mM EDTA) are acceptable, but use of these solutions must be recorded in the Sample Submission Form. Please note that the sample should be free of additives or impurities such as ethanol, salts, phenol, or EDTA concentrations greater than 1mM.

For Affymetrix SNP 6.0 Services:

1. Asuragen uses the Affymetrix recommended labeling method for best performance with intact genomic DNA samples.
2. DNA must be RNA-free.
3. Acceptable storage solutions include TE (10 mM Tris, 1 mM EDTA) or nuclease-free water. The storage solution must be recorded in the Sample Submission Form.

Affymetrix SNP 6.0 Services for intact genomic DNA; (target prep# CA0092):

1. Recommended: > 1 µg DNA of concentration ≥ 100 ng/µL
2. Required: 750 ng of concentration ≥ 50 ng/µL (including QC metrics)

NGS: QuantideX[®] Pan Cancer panel (target prep# CA0174):

1. Recommended: >500ng quantified fluorometrically
2. Required: 20ng of concentration ≥ 5 ng/µL
3. Acceptable storage solutions include TE (10 mM Tris, 1 mM EDTA) or nuclease-free water. The storage solution must be recorded in the Sample Submission Form.

Sample Input Requirements: Nucleic Acid Isolations From Cell Lines, Fresh Frozen Or Preserved Tissue, Blood, And Other Biofluids

For all services:

1. RNA yield is primarily dependent on the tissue type and mass (or volume) but is often affected by disease status, chemical treatment, media, preservatives, and tissue handling.
2. RNA integrity is dependent on a wide range of factors in the precise handling of biological materials from collection to RNA isolation. Elimination of the activity of endogenous endo- or exonucleases is vital to preserving RNA integrity, and typically depends on treatment with proper preservative or freezing upon tissue collection.
3. RNA purity is dependent on the quality of the RNA isolation method used, the tissue type, fixation methods, and sample degradation.
4. NOTE: whether from an “experimental” or “normal” population, there is naturally wide variation in yield, degradation mechanisms, and contaminants. Asuragen has performed RNA isolation from thousands of samples, hundreds of tissue types, and dozens of different formats, and we practice methods optimized and tested for maximum yield, integrity, and purity, but we cannot guarantee the yield, integrity, or purity from any given experimental sample.

5. **Unless noted otherwise, all standard RNA isolation methods and custom biofluid RNA isolations are optimized to recover miRNA**
6. When working with biological samples, it is best to work quickly and to use a wet ice bath to ensure that all labeled tubes and containers are chilled. Prior to chilling of collection tubes, ensure that they have been labeled with the Sample ID using a permanent marker or properly rated labels for -80°C storage.

Eukaryotic or Prokaryotic Cell Lines, Total RNA Isolation (#CA0041):

1. After harvest and quantitation, cell lines should be lysed immediately in a guanidinium isothiocyanate (GITC)-containing lysis buffer, then snap-frozen in liquid nitrogen.
2. The recommended range of sample volumes is 1 mL of lysis buffer for 10⁶ cells up to 5 mL of lysis buffer for 10⁷ cells. Single-sample volumes of lysate greater than 5 mL may be processed for an additional charge.
3. Samples should be shipped in primary and secondary containment on dry ice (see Shipping Instructions).

Fresh Frozen Tissue, Total RNA Isolation (#CA0009):

1. Input tissue amount: 1 mg (such as needle biopsies) to > 1 g.
2. Tissue recovered from donor should be transferred to a pre-labeled, pre-chilled storage container.
3. Snap-freezing in liquid nitrogen is recommended for preservation of RNA profiles from tissue, and cutting the tissue into smaller pieces ensures thorough and rapid freezing.
4. If possible, tissue mass should be measured and recorded prior to freezing. Single sample masses > 5 g may be processed for an additional charge.
5. Frozen tissue should be maintained at -80 °C until shipped in primary and secondary containment on dry ice (see Shipping Instructions).
6. Single RNA isolations from tissue, using manual procedure, will accommodate up to 1 g of tissue. Processing of samples in excess of 1 g will require additional isolations for additional cost.
7. Automated isolation procedure is also available – please inquire.

Preserved Tissue (FFPE, Formalin, or RNAlater[®]/RNARetainTM) RNAlater[®] or Formalin Preserved Tissues, Total RNA Isolation (#CA0009):

1. Input tissue amount: 1 mg to > 1 g.
2. Single RNA isolations from tissue, using manual procedure, will accommodate up to 1 g of tissue. Processing of samples in excess of 1 g will require additional isolations for additional cost.
3. The rate of RNA protection for these methods is dependent on tissue size and type due to different penetration rates. Consequently, Asuragen recommends qualifying the procedures used with these reagents in a setting and with sample types and sizes representative of the final use.
4. Samples should be shipped in primary and secondary containment on wet ice.

FFPE samples, Total RNA Isolation (#CA0008):

1. Acceptable sample formats are blocks, slides (unstained and uncovered), or sections.
2. H&E staining, pathology assessment, and macrodissection for tumor enrichment are services available for additional cost for samples submitted as blocks or slides. For H&E staining, a single slide of 3 to 5 µm thickness is required. Alternatively, Asuragen can accept H&E stained slides or electronic images of H&E stained slides for pathology assessment.
3. Up to 80 µm total thickness per sample (slices of 5 to 20 µm thickness each) should be shipped in primary and secondary containment at ambient temperature in an insulated shipping container (see Shipping Instructions). The number and thickness (µm) of sections being shipped in each tube should be recorded.

4. More than 80 µm total thickness per sample (up to 640 µm total thickness) can be processed for an additional charge.
5. The amount of time used to fix the samples and the composition of the fixative used for this purpose should be included in the comments section on the Sample Submission Form.
6. Asuragen recommends characterizing the tissue area and histology adjacent to the slices sent for RNA isolation to better estimate potential RNA yield and enable correlation with conventional pathology assessment.
7. While it is impossible to predict RNA yield from FFPE tissue with great accuracy, Asuragen can provide guidance on RNA yield per tissue volume (area x thickness) for a wide array of common human tissues.

Whole Blood (PAXgene) RNA Isolation, with miRNA (#CA0045) Whole Blood (PAXgene) RNA Isolation, with miRNA, automated (#CA0084) Globin Reduction (GLOBINclear) for Whole Blood (#CA0022):

1. Blood (2.5 mL per sample) should be collected directly into a PAXgene[®] tube via butterfly or by syringe. Following collection, the tube should first be inverted vigorously 10 times or more to ensure proper mixing of the preservative agent and then immediately placed into a -80 °C freezer.
2. The box to be used for shipment should hold 3 times the volume of dry ice as compared to the combined volume of the tubes/bottles. Dry ice should be packed in the bottom of the box before adding samples. Once samples are added, more dry ice should be added to completely surround the tubes in all directions. For international shipments, samples should be packed in 25 lb of dry ice.

Custom RNA or DNA isolations (#CA0057 for custom RNA isolations) Biofluids (plasma, serum, CSF, saliva, urine, or other):

1. RNA (and DNA) isolations from biofluids other than blood are performed on a custom basis by experienced personnel using validated equipment. However, Asuragen cannot guarantee the quality of RNA from any customer-submitted sample, and the customer accepts the risk that yield and quality of RNA from these sample types may be quite low (15-20 ng/ml human plasma or serum as a conservative estimate).
2. RNA yields from biofluids are typically below the reliable limit of detection for NanoDrop spectrophotometry, and as such, Asuragen does not perform routine QC on RNA isolated from biofluids to avoid consumption of already very limiting sample.
3. Biofluids such as plasma, serum, cerebrospinal fluid (CSF), saliva, and urine should be collected and frozen preferably in liquid nitrogen, although -20 °C is acceptable. Frozen samples should be shipped in appropriately labeled sample vessels, surrounded by dry ice, in primary and secondary containment (see Shipping Instructions).
4. Recommended single sample volumes for compatible Asuragen downstream analysis services are at least 1 mL per sample.

Custom DNA isolations (#CA0025):

1. In general, samples to be submitted for DNA isolations should be prepared according to the above guidelines for RNA isolations from each sample type.
2. Exceptions are eukaryotic and prokaryotic cell lines.
3. For total DNA isolations from cell lines, frozen cell pellets (preferably snap frozen in liquid nitrogen) or homogenates in Qiagen RLT lysis buffer should be shipped in primary and secondary containment on dry ice (see Shipping Instructions)

Data requirements: Informatics services

For Informatics Services, the following minimal information is required for data that was generated outside of Asuragen:

1. All raw data file IDs listed in the column “Customer’s Tube Name or Identifier” in Sample Submission Form
2. Association of the file IDs with appropriate sample groupings in column “Factor1” and “Factor2” (if necessary)
3. A description of the experiment, including platform, sample and replicate designations, and QC summaries
4. A detailed statement of work will be created and approved by the customer prior to beginning a custom data analysis project.
5. Raw data files should be submitted on a DVD/CD or hard drive.

Shipping Instructions

1. Ship only on Mondays, Tuesdays, and Wednesdays. Asuragen cannot accept risk due to delays in shipment of samples.
2. Samples should be sent in sufficient dry or wet ice to ensure that they remain frozen until received by Asuragen.
3. For dry ice shipments, ship in an insulated container with enough dry ice for 3-5 days (typically 3 lb for small shipments).
4. Appropriate package notices must be included for dry ice shipping and shipment of known or potential biohazards.
5. Include your Job Number (provided at the time of your quotation) on the mailing label.
6. Please contact your courier or Asuragen for information if you are uncertain about the regulations for shipping various materials.
7. Please ensure that your purchase order and Sample Submission Form have been sent to Asuragen Laboratory Operations before shipping your samples.

Regardless of sample receipt, your project will not be initiated until your electronic Sample Submission Form and Purchase Order have been received by Asuragen.

Please use the following shipping address, referencing your Job Number:

Asuragen, Inc.
Attention: Asuragen Laboratory Operations (Job #_____)
Suite 100
2150 Woodward St.
Austin, TX 78744

Please also include the following e-mail address in your shipping courier’s electronic shipment notification update: serviceorders@asuragen.com.

Checklist for completion of sample submission:

1. The exact shipping address: Asuragen, Inc., Attention: Asuragen Laboratory Operations (Job #_____), Suite 100, 2150 Woodward St., Austin, TX 78744. (Your Job Number will be provided to you by Asuragen personnel.)

2. Service Provider Agreement or Terms and Conditions with Quotation – signed by the authorized representative and sent to the Asuragen Laboratory Operations Customer Service department via fax # (512-681-5202).
3. Purchase Order or Credit Card Authorization that references the quote provided to the Asuragen Laboratory Operations Customer Service department.
4. Sample Submission Form in an electronic format sent to serviceorders@asuragen.com – names and physical details of the submitted samples.
5. Statement of Work (typically included in the quote) provided to the Asuragen Laboratory Operations Customer Service Department.
6. If you are providing Affymetrix GeneChips® or other materials from a third party vendor, direct these materials to the shipment address above, referencing your Job Number or Quotation. Asuragen does not accept shipments of GeneChips directly from customers without written authorization from Affymetrix since this would invalidate the Affymetrix warranty. Please contact your Business Development Manager for help with obtaining required authorization to transfer Affymetrix GeneChips® you have previously purchased to our facilities. Please contact us at support@asuragen.com if you have any questions.