



MERCURIUS™

**Total BRB-seq  
Service**

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**Sample Submission Guidelines**



# Total BRB-seq for 96-well Plate Format

## Sample submission guidelines at a glance

1. Transfer the RNA samples to a 96-well PCR plate following the instructions below and store them at -80°C before shipment.
2. Fill in the Sample Submission Form (**SSF**) and **check all the boxes** in the Sample submission checklist below; send both files to [orders@alitheagenomics.com](mailto:orders@alitheagenomics.com). Please be aware that any inconsistency may lead to delays or additional fees.
3. Request the **shipping address** from your sales specialist.
4. Ship the samples on dry ice, ensuring the plates are placed between layers of dry ice to maintain a consistent freezing temperature throughout transit. Please provide us with the shipment tracking number.

## Sample submission checklist

- Total BRB-seq service is optimized for efficient rRNA depletion in **human, mouse, and rat** RNA samples. For use with other species, please get in touch with us at [info@alitheagenomics.com](mailto:info@alitheagenomics.com) to discuss compatibility.
- The Sample Submission Form (SSF) must be filled out correctly with a unique sample ID. Consider adding a suffix for technical replicates (e.g., XX\_rep1, XX\_rep2, etc.). Ensure that the SSF provides information about all the shipped samples
- Avoid having randomly distributed samples across the plate layout (e.g., in A01, A06, B03, G10-12, etc.).
- The sample volume should be **15 µL** per well **and identical for all samples** (!).
- The concentration of all samples is uniform, and the A260/230 ratio is assessed and provided in the SSF.
- The **minimum number** of samples in each group (to be pooled together) is **16**.
- Samples are provided in the 96-well RNase/DNase-free PCR plate. **Samples in tubes cannot be processed.**
- Plates are labeled with the same Plate ID as indicated in the SSF.
- Plates are well sealed with an adhesive and a temperature-resistant seal (aluminum is ideal).

## Essential considerations for input material

### Sample quantity and integrity

- The tested range of total RNA amount is 100 pg – 100 ng (per well).
- The recommended amount is 10-100 ng of total RNA per well.
- The volume should be 15 µL/sample and identical across all samples.
- The recommended RIN number is > 7

### Samples purity

- RNA samples, extracted with TRIzol™, phenol, chloroform, or guanidine, are prone to residual contamination with organic solvents that considerably decrease cDNA yield. Make sure to follow the washing steps of the used protocol.
- To ensure the high purity of RNA, assess the 260/230 ratio for all samples.
- Samples should be free of salts and DNA.
- The 260/230 ratio values should be between 1.8 and 2.2.

### Samples uniformity

- To ensure an even distribution of reads after sequencing, the RNA amount, integrity, and 260/230 values of the starting RNA samples must be as uniform as possible, with a max 10% variation.
- To obtain such uniform amounts, we recommend the following:
  - Use dye-based methods for RNA quantification (e.g., Qubit Quant-iT or RiboGreen).
  - Dilute samples to obtain the same RNA concentration in all wells ( $\pm 10\%$ ).
  - Re-measure the RNA concentration of all samples to confirm uniform concentration.
  - Ensure the 260/230 ratio is between 1.8 and 2.2.

### Batch-effect and sample replicates

- RNA extraction protocol can produce considerable technical variation across the samples; therefore, performing RNA extraction in a single batch is strongly recommended.
- If the differential expression (DE) analysis is planned, the respective RNA samples should be included in the same library. Comparison of samples from different libraries can be biased.
- Including at least 3 (or more) biological replicates is highly recommended for the reliability of the experimental setup.

### Samples preparation

1. Label a new 96-well RNase/DNase-free PCR plate.
2. Pipette the RNA samples into the new 96-well PCR plate according to the filled Sample Submission Form. Follow the column-based direction (column 1, then column 2, etc.).
3. Seal the 96-well PCR plate with an aluminum seal and briefly spin it down.
4. Store the samples at -80°C before shipment.



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