UNIVERSITY OF LIVERPOOL | CENTRE FOR GENOMIC RESEARCH

Sample requirements for the RNA NanoString applications

Required amounts of starting material for the RNA NanoString applications:

Application	Starting material	Sample concentration	Required total quantity
Gene expression	Total RNA	20 - 100 ng/µl	≥150 ng
	Cell lysate	2,000 - 10,000 cells/µl	≥15,000 cells
	FFPE RNA	20 - 100 ng/µl	≥150-300 ng
miRNA	Purified total RNA only	33 - 100 ng/µl	≥150 ng
	FFPE RNA	33 - 100 ng/µl	≥150-300 ng

Gene Expression Assay: For purification of total RNA and FFPE RNA, standard commercially available kits are recommended (Ambion™ and Qiagen™, for example). Samples should be resuspended in purification kit buffers, RNase free water, TE, or Tris buffer and dispensed into RNase free tubes or microtiter plates.

Whole cell lysates should be prepared in a guanidinium-based (GITC) lysis buffer such as Qiagen™ buffer RLT. Avoid making the lysate too concentrated, such that cell lysis can be inhibited and the solution too viscous to pipette effectively. NanoString recommend no more than 1.5 µl of these lysis buffers be used per nCounter XT hybridisation.

For small numbers of initial cells, obtained by flow sorting, for example, NanoString recommend sorting directly into a chemical or detergent-based buffer such as Cells-to-Ct (up to 2,000 cells/µl). Solutions such as RNAlater are not compatible with this application.

miRNA Assay: The nCounter® miRNA Expression Assay requires purified total RNA as input material. NanoString recommend the use of approximately 100 ng of total RNA, as this quantity of input material generates robust signal for most tissue and cell isolates. Total RNA purified from any cell or tissue type may be used in the assay, including formalin-fixed, paraffin-embedded (FFPE) material.

Unpurified lysates may not be used with the nCounter miRNA Expression assay, as the denaturants in the homogenization buffer will inhibit the sample preparation reaction. The quality of the purified RNA is critically important for the nCounter miRNA assay, as residual contaminants left over from lysis and RNA extraction can impact assay performance by inhibiting the enzymatic ligation and purification steps.

Typical lysis or extraction contaminants that can inhibit the assay include: Guanidinium isothiocyanate (lysis buffer), phenol (organic extraction), guanidinium HCl (initial wash buffer) and ethanol (secondary wash buffer).

RNA quality: Purified RNA quality can be evaluated by spectrophotometry by measuring absorbance at 230, 260 and 280 nm. The 260/280 ratio may indicate contamination with proteins, whereas the 260/230 ratio may indicate contamination with organic compounds, such as phenol and guanidinium salts.

NanoString recommend a 260/280 ratio of ≥1.9 and a 260/230 ratio of ≥1.8 for optimal results.

Unless specified in the quote, the QC workflow for samples on the NanoString platform does not include RNA integrity check on the Bioanalyzer, but is restricted to Qubit quantification and volume checks.

Note: The NanoString contains internal ERCC spike-in controls. Please make sure that your experiments do not include any of the ERCC spike-ins.