nCounter Service

Sample type considerations

The starting sample recommendations for nCounter assays have been developed using total RNA purified from a variety of tissues, of which mRNA typically constitutes 5-10% (~5-10 ng in a 100 ng total RNA sample).

Blood samples

Blood samples can be analysed using purified total RNA, unpurified blood lysates or specific blood fractions, such as PBMCs isolated from whole blood. NanoString recommends the use of commercially available kits to collect and purify RNA from starting blood, although kits for other biological fluids such as sputum or urine can also be used. For unpurified RNAs, NanoString recommends collecting blood lysate samples in specialised PAXgene® tubes.

Paraffin-embedded or FFPE

For **paraffin-embedded or FFPE** samples, 5 µm sections can be used, although **performance** is generally **optimal with 10-20 µm thick sections** due to the higher percentage of intact cells in larger sections. NanoString recommends that serial sections be taken for histological or pathological evaluation before and after cutting sections to be used for nucleic acid extraction.

A wide variety of extraction methods can be used to isolate nucleic acids from paraffin-embedded samples. Regardless of the extraction method used, **it is important to quantify and check the RNA quality of the extracted material prior to hybridisation.** See Annex 1 for more information on RNA extraction from paraffin-embedded tissue.

RNA quantification using spectrometry (Nanodrop) and fluorescence (Qubit or similar)

Use a **NanoDrop™** or other spectrophotometer to measure the purity of the RNA sample. NanoString recommends an **A260/A280 ratio of 1.7-2.3** and an **A260/A230 ratio of 1.8-2.3**. In addition, it is recommended to check the amount by a **fluorimetric method** (Qubit Life technologies or similar) to confirm **>20ng/µL.**

Amount required for service (ng RNA of starting sample):

Sample type	Minimum volume (µL)	Minimum concentration (ng/µL)	Minimum TOTAL amount (ng)
FFPE RNA	15	60	900
Total RNA (CodeSets panels <400 genes)	15	30	450
Total RNA (CodeSets panels >400 genes)	15	20	300

In addition, **the quality of the RNA needs to be assessed using a fragment analysis system to measure the fragmentation status of the material if it comes from FFPE tissue**. NanoString recommends that at least **50% of the sample be longer than 200 nucleotides (nt)** for optimal hybridisation performance. RNA samples exhibiting higher levels of fragmentation can be used, but the starting amount has to be increased/corrected.

The appropriate amount can be estimated with the following equation:

100/% sample >200 nt) x 100 ng

The percentage of sample above 200 nt can be estimated in BioAnalyzer/TapeStation or similar. This calculation is a tool to help increase the amount of suitable sample, but not a complete predictor of success (especially for those cases with less than 25% of fragments above 200 nt and with an extremely low concentration (<20 ng/µL).

For samples that do not reach this indicated concentration, concentration using columns (such as Amicon Ultra YM-3, 3000 kDa MWCO by Millipore) can be attempted.

Some panels allow less total starting RNA (from 10-50ng) and have available an additional reagent "Low RNA Input kit" to perform an amplification before hybridisation and reading in nCounter. Consult us for this particular case or review the MAN-10046 manual for the use of the nCounter Low RNA Input Amplification Kit.

Always store RNA at -80°C until use/shipping.

NanoString does not recommend a specific RNA extraction kit, but it does recommend to always elute in an elution volume <30 µL and to pass the eluate back through the column, as this may increase the final concentration.

NanoString recommends digestion with Proteinase K for 2-3 hours. Certain fatty tissues (breast, skin, etc.) may require longer digestion, even up to 24 hours more, if the tissue can withstand it.

RNA previously extracted but not treated with Proteinase K can be used; however, contaminants from the preservation method may decrease the efficiency of the assay. The DNAase treatment step is not necessary but is highly recommended to remove genomic DNA from samples that may cause an overestimation in the amount of starting RNA and lead to reduced detection of genes that are less expressed.

Control de calidad del ARN

15 µL shipment at 20-100ng/ µL according to DV200.

- *Concentration: >20 ng/µL (fluorimetric method).*
- *Purity: A260/A280 and A260/230 ratios between 1.7 - 2.3 (spectrophotometer).*

If FFPE, DV200 value (% >200nts) by fragment analyser.

Annex 1: Additional recommendations for the extraction of FFPE tissue material

48 mm^2 is the minimum area of starting material for 5 µm thick sections to obtain an RNA concentration of 10 ng/µL and 50 ng, although an increase in the number of tissue sections improves the likelihood of obtaining the recommended RNA concentrations for these assays. See Table 1 for minimum recommendations for number of sections and sizes.

Table 1. Minimum number of sections according to tissue area and thickness

Tumour surface area (mm^2)	Minimum number of sections (assuming 5µm** of tissue)
2-4	12
5-7	8
8-15	6
16-23	3
24-27	2
>48	1

Table 1 ** Samples with tumour surface area <50% (by total surface area) should be macrodissected to remove non-tumour tissue. Similarly, non-tumour or normal lymphoid structures containing non-contiguous anatomical lymphoid structures should be removed by macrodissection prior to RNA extraction.