

BAP.04800 Nucleic Acid Quantity and Quality Determination Phase II

The quantity and quality of nucleic acids are determined, when appropriate.

NOTE: The quantity and quality of nucleic acids (DNA or RNA) must be measured prior to use in a procedure whose success depends on accurately determining the quantity, concentration, integrity, and/or purity of the nucleic acids. Techniques commonly used to assess nucleic acid quantity and/or quality include electrophoresis, UV/VIS spectrophotometry, and fluorescence spectroscopy.

Standard measure for DNA purity is A260/280 ratio of 1.6 to 2.0. Values less than 1.6 are indicative of protein contamination and values of >2.0 are indicative of RNA contamination. RNA should have A260/280 ratio of greater than 2.0. Analytical measures of nucleic acids include, but are not limited to: A260/280 spectrophotometric ratio, RNA-specific measures, double-stranded DNA (dsDNA), or integrity by agarose gel electrophoresis. RNA integrity assessments should be determined if such a quality indicator would exclude samples from specific downstream methodologies.

RNA in specimens is highly labile because RNase is ubiquitous and difficult to inhibit. For human RNA targets, RNA quality must be assessed. However, depending on the target, it may not be necessary for all specimens to be assessed for RNA quality. RNA quality is not assessed, for example, for many types of viral RNA targets; however, the false negative rate must be recorded.

Evidence of Compliance:

- ✓ Records of nucleic acid quantity and/or quality determination

REFERENCES

- 1) Tsui NBY, Ng EKO, Lo YMD. Stability of Endogenous and Added RNA in Blood Specimens, Serum and Plasma. *Clin Chem* 48:1647-1653, 2002
- 2) Farrell R. Gel electrophoresis based assessment of cellular RNA quality may also be used (RNA Isolation Strategies). In: RNA Methodologies: A Laboratory Guide for Isolation and Characterization. Academic Press, 1998
- 3) Clinical and Laboratory Standards Institute. *Diagnostic Nucleic Acid Microarrays: Approved Guideline*; CLSI Document MM12-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2006.

BAP.04900 Human/Non-Human DNA Phase I

When the downstream application requires an estimation of the ratio of human versus non-human genomic DNA in the specimen, the human/non-human DNA quantity is measured.

BAP.05100 Neoplastic Cell Content Phase II

For paraffin-embedded tumor specimens from which DNA or RNA is extracted for analysis (eg, microsatellite instability, KRAS or KIT analysis), there is a record of histological assessment of neoplastic cell content.

NOTE: In addition to confirming the presence or absence of neoplastic cells by a qualified pathologist, it may be necessary for some assays to estimate and consider neoplastic cellularity in relation to the lower limit of detection of the assay.

A corresponding H&E section from the same tissue block used for nucleic acid extraction may be used to assess sample adequacy. Alternatively, a stain such as toluidine blue may be used to stain the slide that is being used for nucleic acid extraction. When assessment of sample adequacy is performed outside of the testing laboratory, a record of such assessment must accompany the sample.

This requirement is applicable to all molecular methods for the detection of sequence variants (eg, Sanger sequencing, NGS, PCR).

BAP.05125 Ribonuclease-Free Conditions Phase I