**FFPE DNA Extraction and Purification for OncoScan® CNV Assay**

The protocol found in Appendix E, FFPE DNA Extraction Protocol for OncoScan® CNV Assay is required and critical to generate good quality samples for the OncoScan® CNV Assay. DNA must be eluted using the elution reagent provided in the recommended kit (ATE buffer). DNA MUST NOT BE eluted in Water.

**Other optional tested elution reagents:**

1x TE Buffer with low EDTA at pH 8.0 (with 10 mM Tris at pH 8.0 and 0.1 mM EDTA)

Please refer to the QIAamp DNA FFPE Tissue Kit protocol for more information on the QIAGEN webpage given below.

http://www.qiagen.com/Products/Catalog/Sample-Technologies/DNA-Sample-Technologies/Genomic-DNA/QIAamp-DNA-FFPE-Tissue-Kit#resources

**dsDNA Quantification Protocol for OncoScan® CNV FFPE Samples**

Perform either of the two Affymetrix recommended and tested dsDNA quantitation protocols to measure the concentration of the eluted FFPE DNA. These protocols are provided in:

**Appendix F**, PicoGreen® dsDNA Quantification Protocol for OncoScan® CNV Samples on page 127

**Appendix G**, Qubit® dsDNA Quantification Protocol for OncoScan® CNV Samples on page 130,

IMPORTANT: Affymetrix strongly recommends using the QIAamp® DNA FFPE Tissue Kit protocol for purifying DNA from FFPE Blocks that will be used in the OncoScan® CNV Assay. For improved DNA yields, we also recommend a modification to the QIAamp DNA FFPE Tissue Kit protocol. The modified procedure adds a heating step at 98°C for 15 minutes to improve the tissue digestion process to release DNA from tissue sections.

NOTE: Affymetrix highly discourages the use of any sample preparation that is not silica based in OncoScan® CNV FFPE Assay.

IMPORTANT: It is mandatory to determine the sample concentration using a dsDNA specific quantification method. We strongly recommend using either Quanit-iT™ PicoGreen Assay or Qubit dsDNA Quantification, both by Life Technologies, as both of these methods have been validated for use in the OncoScan® CNV FFPE Assay. Sample concentration determined by UV absorbance or NanoDrop must not be used at all in this assay.

NOTE: There are other dsDNA quantitation kits available that may deliver results different than the above mentioned kits. Hence, Affymetrix strongly recommends not using any kit other than the ones recommended above.

**Sample Normalization (dilution to a working concentration of 12 ng/μL) at least 15 ul**

All genomic DNA samples should be normalized to a single concentration of 12 ng/μL using low EDTA 1X TE buffer (10mM Tris, 0.1mM EDTA, pH8.0). The positive control DNA that is included in the OncoScan® CNV FFPE Assay Kit is already normalized to a working concentration of ~12 ng/μL.