

Human Residual DNA Quantitation from Direct Real Time PCR

Ivy Fine Chemicals, **Catalog No. hDNA36001**, 100 Reactions
Laboratory Use Only



Digestion Buffer (1500 uL for 100 rxn), 2x PCR Enhancer (10 mL for 100 rxn), 20x Real Time PCR Primers and Probe (100 uL for rxn, Reporter FAM and Quencher Black Hole)

Introduction:

Residual human DNA is a host cell-derived impurity for gene therapy and must be tested and controlled for human use. Often a DNA purification through sodium iodide extraction, Qiagen column, magnetic beads or phenol chloroform extraction has been required for a successful PCR reaction. The complex procedures and DNA loss during binding, precipitation and washing have caused significant variabilities among scientists and laboratories. Here our optimized direct real time PCR kit allows an efficient protein digestion, and a direct and simple gene quantitation by real time PCR or digital PCR.

No sample separation and purification are necessary from our kit. No special Lab skills are required for a consistent DNA quantitation with short hands-on time and automation potential.

Procedures:

1. Dilute human DNA standard and samples (process intermediates and drug substance) in molecular grade water
2. Set up Digestion as follows:

Materials	Volume per Reaction
Digestion Buffer	15 uL
Proteinase K (Invitrogen Catalog 25530-049, 20 mg/mL)	5 uL
Diluted human DNA Standard or Sample	80 uL
60°C for 30 minutes and 95°C for 10 minutes	

Note: Digestion Buffer and Proteinase K can be premixed at 15:5 ratio and loaded to each reaction at 20 uL. Volume can be scaled up depending on the number of reactions in your experiment.

3. Add 100 uL 2x PCR Enhancer to each reaction
4. Real time PCR or digital PCR:

Real Time PCR	Digital PCR
10 uL of 2x qPCR Master Mix	5 uL of 4x dPCR Master Mix
1 uL of 20x Primers and Probe	1 uL of 20x Primers and Probe
9 uL above treated human DNA standard or sample	9 uL above treated human DNA standard or sample
N/A	5 uL molecular grade water

Run instrument default PCR program (extension at 60°C)

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