Direct Real Time PCR Kit without DNA Purification (# BP2310)

Ivy Fine Chemicals, Catalog No. BP2310, 50 Reactions
Laboratory Use Only

2x Digestion Buffer (750 uL for 50 rxn), and 20x PCR Enhancer (75 uL for 50 rxn) from Ivy Fine Chemicals, 1879 Old Cuthbert Rd, Suite 23, Cherry Hill, NJ 08034

DNA purification through sodium iodide extraction, Qiagen column, magnetic beads or phenol chloroform extraction has been absolutely required for a successful PCR reaction. The complex procedures and DNA loss during binding, precipitation and washing has 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 PCR or real time PCR. No sample separation and purification are necessary from our kit. No special Lab skills are required for a consistent DNA quantitation. No need to make a significant change of your sample dilution and standard ranges. No need to make calculation conversions since samples and standards are processed through the same digestion and real time PCR procedures. Significant reduced hands-on time (about 30 min) for the testing and be replaced by complete automation.

This method may apply to host residual DNA impurity quantitation for monoclonal antibody analytics, manufacture and clinical serum samples for gene and cell therapy.

Recommended Protein Digestion:

BSA solution: completely dissolve BSA in molecular grade water to make 6 mg/mL, aliquot and store in freezer. Once thawed for use, don't reuse thawed BSA and recommend to discard remaining. BSA solution is used to add protein stress to DNA standards and to match and represent sample matrix.

Samples: dilute samples to 6 mg/mL in molecular grade water. You may increase or reduce sample protein concentration depending upon the sample matrix impact on your final DNA quantitation and assay variability. If you adjust your sample protein concentration, you need to make corresponding change of BSA concentration as well.

Spiked Samples: add concentrated DNA prepared in BSA solution described below for spiking purpose. If you do see potential BSA interference, you may need to use water diluted DNA standards for your spiking purpose.

DNA Standards: make serial dilution of your DNA standards in 6 mg/mL BSA.

Samples and Standards: transfer 15 uL of each sample or standard to a 96-well PCR plate. Add 9 uL of 20 mg/mL Proteinase K solution (e.g., Invitrogen catalog 25530-049) to 741 uL of 2x Digestion Buffer and mix by gentle inverting and immediate use. Use multiple channel pipette to transfer 15 uL 2x Digestion Buffer containing Proteinase K to each well, followed by gentle pipetting a few times to mix. Seal the PCR plate with a PCR plate seal and run 60C for 120 min and 95C for 5-10 min in a real time PCR instrument. Some instrument software may not allow you to run 120 min long and you can break the 120 min into several cycles, e.g., some at 60C and some at 59C.

PCR or Real Time PCR: use a multiple channel pipette and transfer 10 uL digested and heat inactivated samples, spiked samples and standards into a new PCR plate, followed by 20 uL real time PCR mix containing 1.5 uL of 20x Primers and probe, 1.5 uL of 20x PCR Enhancer, 15 uL 2x PCR master mix and 2 uL molecular grade water. Seal the plate, brief vortex and a quick spin. Run your PCR cycling program for the quantitation of DNA.