EZ DNA Capture for Real Time PCR Quantitation

Ivy Fine Chemicals, Catalog # **DNA82034**, 96 DNA Capture and PCR Reactions
Store at 4°C for 12 months, Research Use Only

Provided with Kit:	**Not Provided:**
• One 96-Well DNA Capture (Streptavidin) and qPCR Reaction Plate • 2x Digestion & Binding Buffer (1.8 mL) • 1x Washing Buffer (10 mL)	• 20 mg/mL Proteinase K (Invitrogen Catalog 25530-049, or equivalent) • 5'-biotin forward primer (40 uM, customer specific) • 5'-biotin reverse primer (40 uM, customer specific) • Real time PCR probe (5 uM, customer specific)

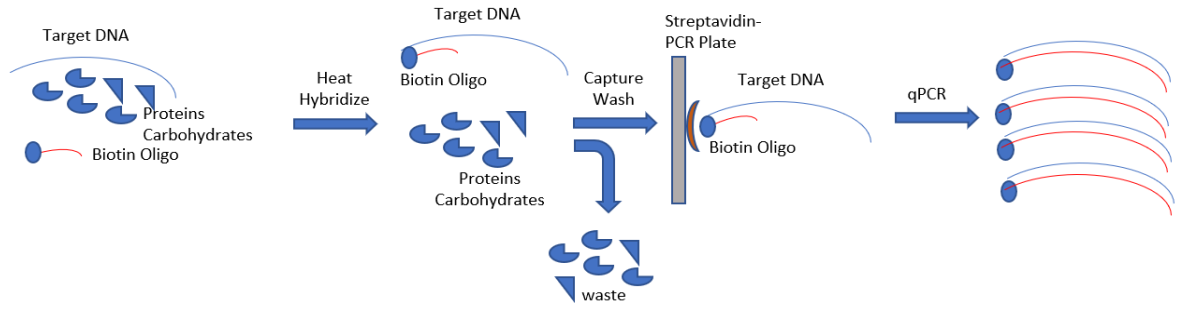
Introduction:

Real-time PCR (qPCR) is a highly sensitive technique for quantifying DNA, which finds extensive applications in DNA bioanalysis for cell and gene (mRNA, viral and vaccine) therapies.

Often sample matrix interferes with qPCR DNA quantitation and must be removed through a DNA sample purification, such as sodium iodide-based purification (Ivy Fine Chemical catalog B48202), Qiagen column or filter plate, magnetic bead etc. All these methods require multiple steps (including washing) and good skills from analysts. Assay variability and DNA loss are common. Instrument investment and long hours in executing experimental steps are a burden to the industry and academic labs.

Here comes an innovative, quick, and easy method: 1) DNA samples are simply incubated with a uniquely formulated buffer containing Proteinase K and specific biotinylated primers of your choice. Protein interference will be reduced, and biotin primers will bind to target DNA. 2) Transfer incubated samples to our Streptavidin-coated Capture PCR plate. DNA target will bind to the Capture PCR plate and remaining interference matrix (including salts and residual proteins) is removed by pipetting out, plate washer or plate flipping on paper towels. Additional one washing may not be needed pending upon the complexity of your sample matrices and needs to be tested during your method development. 3) Real time PCR is performed directly on SAME Capture PCR plate without a need of a DNA elution and transfer to eliminate DNA loss and to reduce assay variability.

Our assay kit is simple and quick. You don't need any special lab skills and don't need to invest in any instruments except real time PCR. Our solution-based assay procedures can be automated at a liquid handler.



Assay Procedures:

1. **Preparation:**
    a. Warm up 2x Digestion & Binding Buffer at room temperature.
    b. Add 80 uL of 20 mg/mL Proteinase K, 120 uL Biotin-Forward primer (40 uM), 120 uL Reverse Biotin-Reverse primer (40 uM) to 1.8 mL 2x Digestion & Binding Buffer (volume can scale up or down proportionally based on your need).
2. **Incubation:**
    a. Add 15 uL of DNA sample or DNA standard to each well of a standard PCR plate (Not the kit Capture PCR plate!).
    b. Transfer 15 uL Digestion and Binding Buffer containing Proteinase K and biotin-primers.
    c. Pipetting or vortex to mix. Brief spin if there is too much air.
    d. Run incubation program: 60°C for 15 min, 95°C for 10 min, 40°C for 5 min, hold at 25°C.
3. **Capture and Real Time PCR Reaction:**
    a. Transfer 20 uL of incubated and heated samples to Capture PCR plate and incubate at room temperature for 30 min (shaking recommended).
    b. Add 100 uL Washing Buffer and mix by shaking.
    c. Remove solution from Capture PCR plate by pipetting, plate flipping on paper towels or aspirating through a plate washer.
    d. Add 20 uL real time PCR mix containing 10 uL 2x Master Mix, 1 uL of probe (5 uM) and 9 uL of water.
    e. Run real time PCR quantitation at default program or an annealing and extension temperature optimized for your specific primers and probe.

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