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# Low Abundance DNA Quantification Kit Product Insert

**Product # 57200**

The amount of DNA that can be extracted from different biological or clinical samples varies greatly. For example, while a few micrograms of DNA could be easily purified from tissues and cells in excess amount (such as from a few milligrams of tissue), many liquid biopsy samples may yield very low amounts of DNA. In fact, samples such as urine or plasma may yield 1 - 100 ng or less DNA per 100 µL of sample. The most commonly used technique for measuring DNA concentration is the determination of absorbance at 260 nm (A260). However, even with the new generation of spectrophotometers, the detection limit of this method is still above 2 - 10 ng per µL. Additional technologies, such as the use of fluorescent nucleic acid stains, has enabled the quantification of DNA at the lower ng or sub-ng per µL range. However, this may not completely overcome the difficulties in quantifying DNA from liquid biopsies where the expected DNA yield could be in the lower pg or sub-pg per µL range.

Norgen's Low Abundance DNA Quantification Kit offers a PCR-based detection procedure to quantify DNA of a wide spectrum of concentrations, including the lower ng per µL, pg per µL and sub-pg per µL range. The kit consists of a specially designed primer mix, that is used in conjunction with the provided 2x Real-Time PCR Master Mix, to amplify human DNA from different types of inputs (such as various liquid biopsies). The unknown DNA is accurately quantified by using a standard curve constructed from the provided DNA Standard on a Real-Time PCR System.

## Specifications

| Component | Product # 57200 (48 Samples) |
|---|---|
| 2X Real-Time PCR Master Mix | 1 mL |
| DNA Quantification Primer Set Mix | 200 µL |
| Quantified DNA Standards[1] | 5 Standards, each 100 µL |
| Nuclease-Free Water | 1.25 mL |
| Product Insert | 1 |

[1]Quantified DNA Standards are provided as 20 ng/µL, 2 ng/µL, 200 pg/µL, 20 pg/µL and 2 pg/µL

## Storage Conditions

Upon receipt, store Norgen's Low Abundance DNA Quantification Kit at -20°C or lower. Avoid multiple freeze-thaw cycles. If needed, prepare smaller working aliquots and store at -20°C or lower.

## Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's Low Abundance DNA Quantification Kit is tested against predetermined specifications to ensure consistent product quality.

## Product Use Limitations

Norgen's Low Abundance DNA Quantification Kit is designed for research purposes only. It is not intended for human or diagnostic use.

**Customer-Supplied Reagents and Equipment**

- Real-time PCR platform with FAM/Sybr Green channels
- DNA Purification Kit
    - The kit is compatible with all DNA purification kits that yield high quality, inhibitor-free DNA
    - **Recommended Purification Kit:** Norgen Biotek's purification kits for DNA isolation, including:
        - Plasma/Serum Cell-Free Circulating DNA Purification Mini Kit - Cat# 55100
        - Urine DNA Isolation Micro Kit - Cat# 18100
- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes

**Warnings and Precautions**

- Follow universal precautions. All patient specimens should be considered as potentially infectious and handled accordingly.
- Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when handling specimens and kit reagents.
- Use sterile pipette tips with filters. Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- As contamination of patient specimens or reagents can produce erroneous results, it is essential to use aseptic techniques. Pipette and handle reagents carefully to avoid mixing of the samples.
- Do not use supplies and equipment across the dedicated areas of i) specimen extraction, ii) reaction set-up and iii) amplification/detection. No cross-movement should be allowed between the different areas. Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- Do not substitute or mix reagents from different kit lots or from other manufacturers.
- The presence of PCR inhibitors may cause invalid results.
- Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated.

# Instructions for Use

## A. Sample Preparation

Purified DNA is the starting material for Norgen's Low Abundance DNA Quantification Kit. The quality of the DNA template will have a major impact on the performance of the quantification test. We recommend the use of Norgen's purification kits for DNA isolation, including **Norgen's Plasma/Serum Cell-Free Circulating DNA Purification Mini Kit (Cat# 55100) and Urine DNA Isolation Micro Kit (Cat# 18100)**. It is highly recommended to perform an RNase treatment on the sample to ensure accurate DNA quantification.

If using a different spin column based sample preparation procedure that includes ethanol-based wash buffers, a column drying step consisting of centrifugation for 10 minutes at 14,000 x g (~14,000 RPM), using a new collection tube, is highly recommended prior to the elution of the RNA. This will help to prevent the carry-over of any ethanol into the purified DNA, as ethanol is known to be a strong inhibitor of PCR. **Ensure that any traces of ethanol from the sample preparation steps are eliminated prior to the elution of the DNA.**

## B. Quantitative PCR Assay Preparation

**Notes Before Use:**

- Before use, suitable amounts of all PCR components should be completely thawed at room temperature, mixed by gentle vortexing or by pipetting, and centrifuged briefly. Put all thawed components on ice.
- Avoid repeated freeze and thaw. If needed, make small aliquots of each PCR component and store at -20°C or lower.
- Work quickly on ice.
- The amount of 2X Real-Time PCR Master Mix provided is enough for up to 96 PCR reactions (48 sample PCR and 48 standard curve PCR).
- The recommended minimum number of DNA samples tested per PCR run is 6.
- Follow the order outlined in Tables 1 and 2 below to prepare the Sample Assay and Standard Curve
    1. Prepare the Sample Assay PCR (Table 1)
    2. Prepare Standard Dilution Series PCR (Table 2)
- To further avoid contamination, add the components to the PCR tubes in the order shown in the tables below (ie: 1) Nuclease-free water; 2) Master Mix; 3) Primer Set; and 4) the Sample DNA or Standards).
- The Real-Time PCR Master Mix contains a fluorescent DNA dye compatible with SYBR Green/FAM detection.

1. Prepare the PCR reaction for sample detection as shown in Table 1 below. The recommended amount of sample DNA to be used is 2.5 µL. However, a volume between 1 and 5 µL of sample DNA may be used as template. Adjust the final volume of the PCR reaction to 20 µL using the Nuclease-Free Water provided.

**Table 1. PCR Sample Assay Preparation**

| PCR Components | Volume Per PCR Reaction |
|---|---|
| Nuclease-Free Water | 5.5 µL |
| 2X Real-Time PCR Master Mix | 10 µL |
| DNA Quantification Primer Set Mix | 2 µL |
| Sample DNA | 2.5 µL |
| Total Volume | 20 µL |

2. Using the DNA Standard dilution series prepared above, prepare five standard curve PCRs as shown in Table 2 below:

**Table 2. DNA Standard Dilution Series PCR Preparation**

| PCR Components | Volume Per PCR Reaction |
|---|---|
| Nuclease-Free Water | 5.5 µL |
| 2X Real-Time PCR Master Mix | 10 µL |
| DNA Quantification Primer Set Mix | 2 µL |
| DNA Standard | 2.5 µL |
| Total Volume | 20 µL |

## C. PCR Assay Programming

1. Program the thermocycler according to the program shown in Table 3 below. Set up plate read of SYBR Green/FAM dye for Cycle 2, Step 3.
2. Run the Real-Time PCR.

**Table 3. PCR Assay Program**

| Real-Time PCR Cycle | Step | Temperature | Duration |
|---|---|---|---|
| ***Cycle 1*** | **Step 1** | **95°C** | **3 min** |
| ***Cycle 2 (40x)*** | **Step 1** | **94°C** | **15 sec** |
| | **Step 2** | **37°C** | **60 sec** |
| | **Step 3** | **72°C** | **60 sec** |

## D. DNA Quantification Assay Interpretation

1. For real-time analysis, using the analysis software of the thermocycler, generate a standard curve using the Ct values of the DNA Standard dilution series. The standard curve can then be used to determine the starting quantity of the sample of interest.

2. If the amount of sample DNA and the amount of DNA Standard dilution series used per PCR reaction are not the same, ensure that the proper volume adjustment is made in order to attain the correct DNA concentration of the sample.

**Example 1 – DNA Sample used in Table 1 is 2.5 µL and DNA Standard used is 2.5 µL**

If observed sample DNA concentration based on Standard Curve is 50 pg/µL, then

$$\textbf{Sample DNA Concentration} = 50\ \text{pg/µL} \times \frac{\text{(Standard Volume, 1 µL)}}{\text{(Sample Volume, 1 µL)}} = 50\ \text{pg/µL}$$

**Example 2 – DNA Sample used in Table 1 is 1 µL and DNA Standard used is 2.5 µL**

If observed sample DNA concentration based on Standard Curve is 50 pg/µL, then

$$\textbf{Sample DNA Concentration} = 50\ \text{pg/µL} \times \frac{\text{(Standard Volume, 2.5 µL)}}{\text{(Sample Volume, 1 µL)}} = 125\ \text{pg/µL}$$

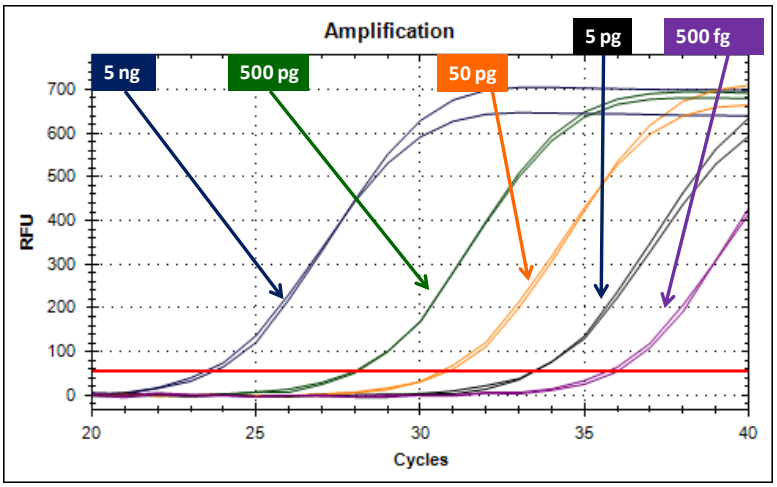


**Figure 1:** A representative qPCR baseline graph showing the successful amplification of a dilution series of Quantified DNA Standard.

| Related Products | Catalogue Number |
|---|---|
| Plasma/Serum Cell-Free Circulating DNA Purification Micro Kit | 55500 |
| Plasma/Serum Cell-Free Circulating DNA Purification Mini Kit | 55100 |
| Plasma/Serum Cell-Free Circulating DNA Purification Midi Kit | 55600 |
| Plasma/Serum Cell-Free Circulating DNA Purification Maxi Kit | 55800 |
| Plasma/Serum Circulating DNA Purification Mini Kit (Slurry Format) | 50600 |
| Plasma/Serum Circulating DNA Purification Midi Kit (Slurry Format) | 51200 |
| Plasma/Serum Circulating DNA Purification Maxi Kit (Slurry Format) | 51300 |
| Plasma/Serum RNA/DNA Purification Mini Kit | 55200 |
| Plasma/Serum Circulating Nucleic Acid Purification Mini Kit (Slurry Format) | 53300 |
| Plasma/Serum Circulating Nucleic Acid Purification Maxi Kit (Slurry Format) | 53400 |
| Urine DNA Isolation Micro Kit | 18100 |
| Urine DNA Isolation Kit (Slurry Format) | 48800 |
| Urine Cell-Free Circulating DNA Purification Mini Kit | 56600 |
| Urine Cell-Free Circulating DNA Purification Midi Kit | 56700 |
| Urine Cell-Free Circulating DNA Purification Maxi Kit | 56800 |

## Technical Support

Contact our Technical Support Team between the hours of 9:00 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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