NUCLEIC dotMETRIC™

G-Biosciences

Abstract

1µl Assay for detecting and measuring DNA, RNA & Oligos.

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Guidelines

INTRODUCTION

The patented NUCLEIC dotMETRIC™ kit provides a quick and accurate method for detecting and measuring DNA, RNA and oligonucleotides. This system is an alternative to spectrophotometry; no expensive equipment or cuvettes are necessary. NUCLEIC dotMETRIC™ uses as little as a microliter of your sample and provides permanent results in minutes.

Two methods in one: NUCLEIC dotMETRIC™ offers two separate protocols for measuring nucleic acid concentration; the concentration of nucleic acid can be measured either by measuring the diameter of nucleic acid spots or comparing the color density of the nucleic acid spots with that of a set of known standards.

To use NUCLEIC dotMETRIC™, dilute samples with dilution buffer, spot 1-5 µl of each sample onto the NUCLEIC Test Strip, develop the strip with NUCLEIC dyes, wash and dry. Using the NUCLEIC dotMETRIC™ scale, dot diameter is measured for accurate determination of nucleic acid concentration. Alternatively, compare the color density of the nucleic acid spots with that of a set of known standard nucleic acid spots.

Samples in the range of 10µg/µl to 1ng/µl can be measured accurately regardless of the isolation method or storage buffer. Oligonucleotides as short as 16 bases, have successfully been measured with NUCLEIC dotMETRIC™ . Measurements are not affected by proteins. The lower detection limit of the system is 1-2ng/µl making NUCLEIC dotMETRIC™ one of the most sensitive methods available for measuring nucleic acids.

ITEM(S) SUPPLIED

Description	Cat. # 786-60	Cat. # 786-61
NUCLEIC Test Strips	50	50
NUCLEIC Dye	30ml	30ml
Nucleic Acid Dilution Buffer	10ml	10ml
dotMETRIC™ Scale	1	1
dotMETRIC™ Standard	1	1
Application Board	1	1
Forceps	1	1
dotMETRIC™ Application Device	-	1
1µl Application Capillary	-	100

STORAGE CONDITIONS

The kit is shipped at ambient temp. Upon arrival, store it at room temperature.

ADDITIONAL ACCESSORIES

The following items are also available separately:

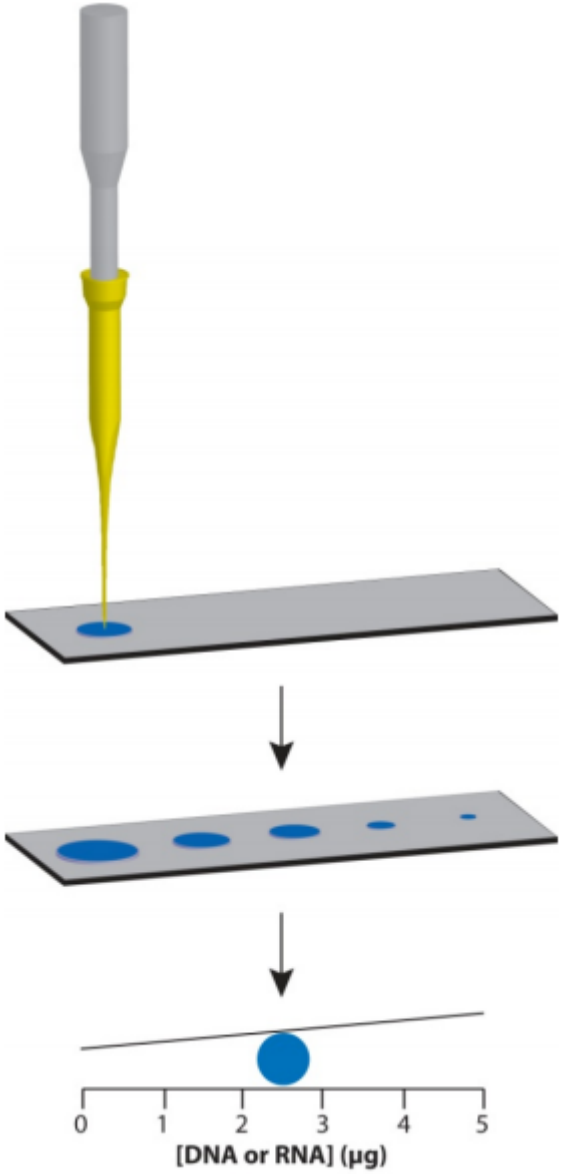
• Application (Pipette) Tips [Cat #786-64]

• dotMETRIC™ Spot Application Device [Cat #786-63]

• 1µl Application Capillary [Cat #786-23]

• Developing Trays [Cat #786-24]

QUICK PROTOCOL



1. Dilute sample in Dilution Buffer 1:1 to 1:10 fold.

2. Apply 1-5µl sample on the Test Strip, either as a free drop or as point of contact capillary action, or both.

3. Develop the Test Strip and determine concentration.

SPOT APPLICATION DEVICE

Due to the above-indicated limitations, we strongly recommend using G-Biosciences' Spot Application Device. The Spot Application Device is easy to work with and generally makes application easy and gives better results

Using Application Device for Applying Nucleic Acid Samples.

1. Perform nucleic acid application in a well-lit area and make sure that you can see the solution rise and flow through the application capillaries.
2. Use forceps for handling the application capillary tips. Place the application tip into the solution mixed with the Dilution Buffer; the solution will rise up into the capillary application tip and fill within 3-5 seconds.
3. Position the application tips in the holes provided in the device and lower the tip until the tip touches the membrane surface. As soon as the tip touches the membrane, the solution will begin to flow in to the membrane.
4. After the samples have been diffused into the membrane and the application tip is empty of the solution, remove the application tip from the device. Same capillary tip can be used for several applications.
5. For applying more than 1µl sample on each spot (2-4µl), apply the sample multiple times
NOTE: Allowing the sample to dry before applying a second time is not necessary.

TROUBLESHOOTING

Problem: Sample concentrations below 0.03µg/µl. When nucleic acid concentrations fall below 0.03 µg/µl. The diameter of nucleic acid spots is no longer large enough to be measured with the dotMETRIC™ scale.

Solution: The lower detection limit of nucleic acid in dotMETRIC™ application averages around 1ng/µl. Take 1-2 µl of sample and prepare serial dilutions in dilution buffer, such as x2, x4, x8, x16, x32, x64.fold. Use dotMetric™ application and spot 1 µl from each dilution. Develop the strip. For concentration determination find the last visible spot at the highest dilution. Multiply the dilution fold of the last visible spot by (1+0.1) ng/ul.

Problem: DNA solution does not flow easily into the Test Strip.

Solution: This is due to large strings of DNA in the solution. Dilute the DNA solution further with the Dilution buffer to lower the DNA concentration below <1µg / µl and after mixing, shear the DNA by vigorously vortexing, sonication, or pipetting.

Problem: Dots are not a uniform circle.

Solution: Take care to maintain the pipette tip in a straight up position while the sample is drawn by capillary action of the NUCLEIC Strip. Samples generally take 5-15 seconds to be absorbed depending on the concentration of nucleic acid. Alternatively, use Spot Application Device.

Problem: The dots are faint and/or grainy in appearance. In some instances dots are surrounded by a fuzzy 'halo'.

Solution: These problems are usually caused by high concentrations of impurities in your samples (i.e., salts, phenol, organic solvents). Samples with high concentrations of nucleic acids can be diluted further with the appropriate dilution buffer and measured again. A second solution is to purify your samples using GET™ CLEAN DNA (Cat. # 786-356 or 786-357) or ethanol precipitation. Removing impurities from your samples is also likely to improve your success with any down-stream applications.

Problem: Samples of known concentration vary consistently from the NUCLEIC dotMETRIC™ scale measurements.

Solution: Personal technique and pipette tip size are the most common reasons for inaccurate measurements. Measuring each sample at different dilutions in dilution buffer several times will normally provide very accurate results. The NUCLEIC dotMETRIC™ scale was calibrated with fixed volume micro capillary tips, using the application device. Use of other tips may influence dot diameter. Use Spot Application Device for better results.

Materials

NUCLEIC dotMETRIC™ Assay 786-60 by G-Biosciences

Protocol

Preparation

Step 1.

For double stranded DNA, use undiluted Nucleic Acid Dilution Buffer. For RNA or oligos, dilute the Nucleic Acid Dilution Buffer 100 fold. i.e. Use 1µl Nucleic Acid Dilution Buffer in 99µl ultrapure water.

Preparation

Step 2.

Dilute samples at least 1:1 with the appropriate Dilution Buffer.

NOTES

Colin Heath 08 Jun 2016

NOTE: For many samples, good results will be achieved by diluting 1:4 to 1:10. Two or more dilutions and spots per sample will increase the accuracy of your measurements.

NOTE: For genomic DNA, after mixing with the Dilution Buffer, pipette several times to shear the

DNA. Unsheared genomic DNA may be difficult to apply on the Test Strip. For better results, dilute DNA to achieve <1µg/µl.

Preparation

Step 3.

Open the NUCLEIC Test Strip box, remove a strip, and peel away the protective sheets using the forceps provided.

Preparation

Step 4.

Attach the white NUCLEIC Strip to the application board with the side magnets.

NOTES

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Either side of the NUCLEIC strip can be used. Labeling can be done with a soft pencil or ballpoint pen.

Application

Step 5.

Apply 1-5µl of the diluted samples to the Test Strip using one of these two methods (or see the Guidelines for using G-Biosciences' Spot Application Device):

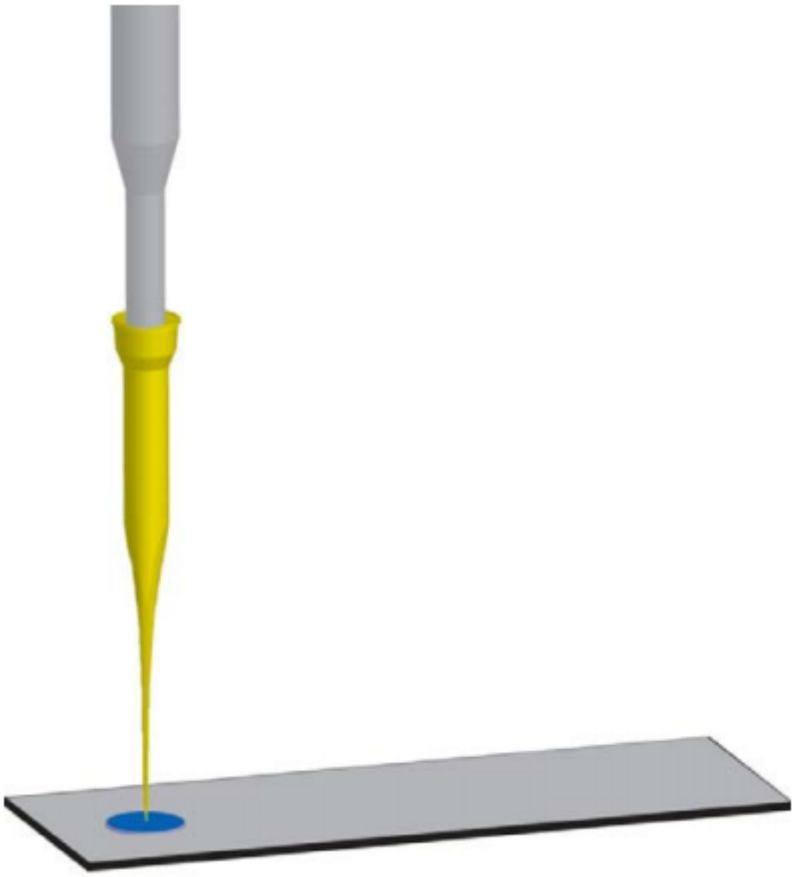
Free-Drop Method Of Application

Squeeze the pipette plunger until a drop is formed at the tip of the pipette tip. Lower the drop until it touches the Test Strip. The nucleic acid solution drop will immediately spread on the Test Strip.

dotMETRIC™ Application Method

Apply sample by point of contact capillary action. Use pipette tips with an outside bore diameterof 0.6-0.7 mm (Cat. # 786-64). Keep the pipette tip straight up and allow the capillary action of the strip to draw the sample from the pipette tip (see figure below). Do not use the plunger to force the solution out.

NOTE: Accurately pipetting 1µl requires skill and caution. A deviation of up to ± 20% could be due simply to pipetting errors. In addition, applying more than >1 µl or multiple spots are sometimes slow



& tedious.

NOTES

Colin Heath 06 Jul 2016

NOTE: If possible, use both methods of application for each sample. It will allow greater flexibility in interpreting results.

NOTE: Make several spots per dilution to increase the accuracy of your measurements.

NOTE: The accuracy of the NUCLEIC dotMETRIC™ system depends on developing a consistent method for applying samples to the NUCLEIC Strip.

Developing Test Strip

Step 6.

Samples do not need to dry before adding NUCLEIC Dye. Place the NUCLEIC strip in a developing tray (Cat. # 786-024) or other small dish.

Developing Test Strip

Step 7.

Apply 0.5ml NUCLEIC Dye directly to the strip.

Developing Test Strip

Step 8.

Incubate at room temperature for 1 minute without shaking or rocking.

DURATION
00:01:00

Developing Test Strip

Step 9.

Discard the NUCLEIC Dye and rinse the strip for 10 seconds in 50ml ultrapure water.

DURATION
00:00:10

Developing Test Strip

Step 10.

Discard the rinse and wash with 50ml ultrapure water for 30-60 seconds or until the blue background disappears. Remove the strip from the second wash as soon as the blue background disappears.

DURATION
00:00:30

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NOTE: Strips do not need to be dry before measuring concentration. Dried NUCLEIC strips can be kept as a permanent record.

Determine Concentration

Step 11.

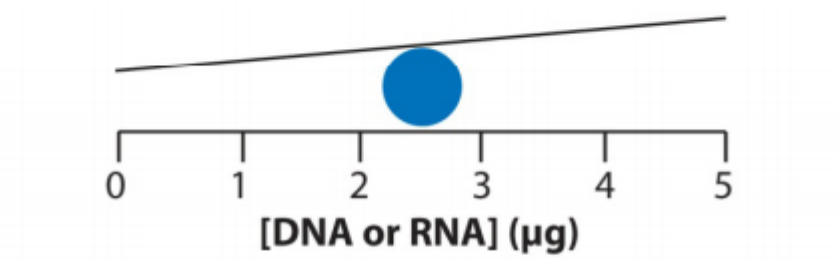
For Free-Drop Method

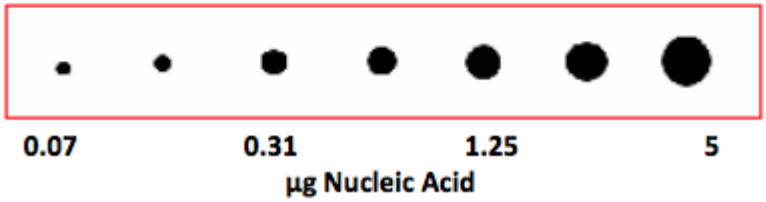
Compare the color of the test sample spot with the dotMETRIC™ Standard provided.

For dotMETRIC Application:

Use the NUCLEIC dotMETRIC™ scale to determine the concentration of the diluted sample. If a dot is not symmetrical measure both the widest and narrowest points of the dot and average. Use the following formula to determine concentration:

$$\text{Sample Concentration} = \text{Dilution Fold} \times \frac{\mu g \text{ nucleic acid in spot}}{\mu l \text{ volume applied}}$$





NOTES

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Example 1µl test sample is diluted with 5µl Dilution Buffer i.e., sample is diluted 6x fold. (Dilution Fold = 6). 2µl of the diluted sample is applied on the test strip, which reads concentration 0.6 µg.

$$\text{Test Sample Concentration} = 6 \times \frac{0.6\mu g \text{ nucleic acid in spot}}{2\mu l \text{ volume applied}} = 6 \times \frac{0.3\mu g}{\mu l} = 1.8\mu g/\mu l$$