NUCLEIC *dot*METRIC™

1 µl Assay for DNA, RNA and Oligos

INTRODUCTION

The patented NUCLEIC *dot*METRIC™ 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 *dot*METRIC™ uses as little as a microliter of your sample and provides permanent results in minutes.

TWO METHODS IN ONE: NUCLEIC *dot*METRIC™ 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 *dot*METRIC™, 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 *dot*METRIC™ 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 *dot*METRIC™. Measurements are not affected by proteins. The lower detection limit of the system is 1-2ng/µl making NUCLEIC *dot*METRIC™ one of the most sensitive methods available for measuring nucleic acids.

ITEM(S) SUPPLIED	Cat # 786-60	Cat # 786-61
NUCLEIC Test Strips	50	50
NUCLEIC Dye	30ml	30ml
Nucleic Acid Dilution Buffer	10ml	10ml
*dot*METRIC™ Scale	1	1
*dot*METRIC™ Standard	1	1
Application Board	1	1
Forceps	1	1
*dot*METRIC™ Application Device	N/A	1
1µl Application Capillary	N/A	100

STORAGE CONDITIONS

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

Additional supplies (Order separately)

The following items are also available separately:

Application (Pipette) Tips [Cat #786-64]	96
*dot*METRIC™ Spot Application Device [Cat #786-63]	1
1µl Application Capillary [Cat #786-23]	100
Developing Trays [Cat #786-24]	2

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.

PROTOCOL

1. Use Nucleic Acid Dilution Buffer with double stranded DNA. For RNA/OLIGO, use diluted Dilution Buffer. Dilute the Dilution Buffer 100-fold (e.g., 1 µl Dilution Buffer mix with 99µl DI water).

Dilute samples at least 1:1 with the appropriate Dilution Buffer. 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. For genomic DNA, after mixing with the Dilution Buffer, pipet several times to shear the DNA. Un-sheared genomic DNA may be difficult to apply on the Test Strip. For better results, dilute DNA to achieve <1µg /µl.

2. Open the NUCLEIC Test Strip box, remove a strip, and peel away the protective sheets using the forceps provided. Attach the white NUCLEIC Strip to the application board (Cat. # 786-22) with the side magnets. Either side of the NUCLEIC strip can be used. Labeling can be done with a soft pencil or ballpoint pen.

3**.** Apply 1-5 µl of the diluted samples to the Test Strip.
Depending on user's preference, samples may be applied by two methods. You may choose either one of the methods. However, if possible, use both methods of application for each sample. It will allow greater flexibility in interpreting results.

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

The accuracy of the NUCLEIC *dot*METRIC™ system depends on developing a consistent method for applying samples to the NUCLEIC Strip.

Free-Drop Method Of Application

Squeeze the pipeter plunger until a drop is formed at the tip of the pipet-tip. Lower the drop until it touches the Test Strip. The nucleic acid solution drop will immediately sprea

***dot*METRIC™ Application Method**

Apply sample by point of contact capillary action. Use pipet tips with an outside bore diameter of 0.6-0.7 mm (Cat. # 786-64). Keep the pipet 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 pipeting 1µl requires skill and caution. A deviation of up to ± 20% could be due simply to pipeting errors. In addition, applying more than >1 µl or multiple spots are some times slow & tedious.

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

USING SPOT APPLICATION DEVICE

Using Application Device for Applying Nucleic Acid Samples.
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.

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.

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.
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.

For applying more than 1µl sample on each spot (2-4 µl), apply the sample multiple times (**NOTE**- allowing the sample to dry before applying a second time is not necessary).

DEVELOPING TEST STRIP

4. Samples do not need to dry before adding NUCLEIC Dye. Place the NUCLEIC strip in a Developing Tray (catalog # 786-024) or other small dish and apply 0.5 ml of the dye on top of the strip. Let the dye stand 30-60 seconds. Do not shake or rock the strips at this point. Pour or pipette off dye. Do not return used dye to reagent bottle.
5. Wash twice with 50 ml of deionized water. The first wash should be quick (5-10 seconds) and wash away excess dye. Remove the strip from the second wash as soon as the blue background disappears (30-60 seconds). Strips do not need to be dry before measuring concentration. Dried NUCLEIC strips can be taped to your notebook as a permanent record.

DETERMINE CONCENTRATION

For Free-Drop Method

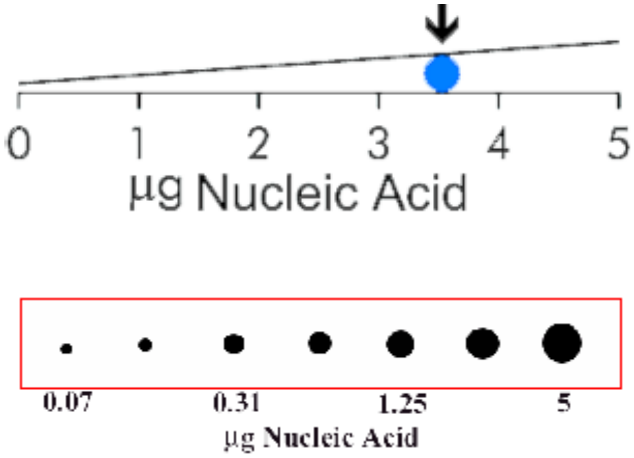
Compare the color of the test sample spot with that of the *dot*METRIC™ Standard provided with the kit.

For *dot*METRIC Application:

Use the NUCLEIC *dot*METRIC™ 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:

Sample Concentration

= Dilution Fold X [µg nucleic acid in spot / µl volume applied]



EXAMPLE-

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

Test sample concentration = 6 x [0.6µg nucleic acid in spot /2 µl] = 6 x 0.3 µg /µl = 1.8 µg /µl

Trouble-shooting

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 *dot*METRIC™ 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 pipeting.

Problem: Dots are not a uniform circle.

Solution: Take care to maintain the pipet 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 remeasured. A second solution is to purify your samples using pinkCLEANUP™ (Cat. #786-87) 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 pipet 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.

RELATED PRODUCTS

1. ***Pre-Diluted Protein Standard (Cat# 786-114):*** *BSA protein standard in an easy-to-use pre-diluted format, supplied as 6x5ml standard ranging from 0.1-1mg/ml.*
2. ***Protein dotMETRIC™ – 1µlProtein Assay: (Cat# 786-20/21):*** *For sample economy and rapid estimation of protein using a test strip. Simply apply 1µl protein solution on the test strip, develop test strips in 8 minutes, and measure the diameter of protein spot on test strip for determination of protein concentration. Protein dotMETRIC™ assay is resistant to reducing agents, detergents and shows little or no protein-to-protein variation.*

NOTE: *For other related products, visit our web site at www.GBiosciences.com or contact us.*