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# Nucleic Acid Quantification

## NUCLEIC dotMETRIC™

Teacher's Guidebook

(Cat. # BE-318)



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## **MATERIALS INCLUDED**

This kit has enough materials and reagents for 50 experiments.

- 1 pack Nucleic Test Strips (50)
- 1 bottle Nucleic Dye
- 1 bottle Nucleic Acid Dilution Buffer
- 1 *dot*METRIC™ Scale
- 1 *dot*METRIC™ Standard
- 1 Application Board
- 6 Forceps
- 50 1.5ml Centrifuge Tubes

## **SPECIAL HANDLING INSTRUCTIONS**

- All reagents can be stored at room temperature.

## **ADDITIONAL EQUIPMENT REQUIRED**

- DNA Samples
- Application Tips (1-10µl pipette tips with an outside bore of 0.6-0.7mm)
- *dot*METRIC™ Spot Application Device (Cat.# D217) & Application Capillaries (Cat.# A113) or 1µl Pipette
- Developing Trays

## **TIME REQUIRED**

- **Day 1:** 30 minutes

## OBJECTIVES

- Understand the principle of determining nucleic acid concentrations.
- Understand nucleic acid measurement with UV absorption.
- Use an alternative nucleic acid quantification assay to UV absorption.

## BACKGROUND

The quantity of nucleic acids (DNA, RNA and oligos) used in many molecular biology applications is often crucial and therefore a reliable method is required to measure the amount of nucleic acids.

The most commonly used method for nucleic acid measurement is UV spectrophotometry. Both RNA and DNA efficiently absorb UV light making it possible to measure as little as 2.5ng/μl. The nitrogenous bases in the nucleic acids have an absorption maximum at 260nm. When a 40μg/ml solution of RNA is measured in a 1cm quartz cuvette the absorption is equal to 1, where as a 50μg/ml solution of DNA would give an absorption of 1. The concentration of RNA or DNA in an unknown sample can be calculated with the following equations:

$$\text{RNA concentration } (\mu\text{g/ml}) = (\text{OD}_{260}) \times (\text{Dilution Factor}) \times ((40\mu\text{g RNA/ml})/(1 \text{ OD Unit}))$$

$$\text{DNA concentration } (\mu\text{g/ml}) = (\text{OD}_{260}) \times (\text{Dilution Factor}) \times ((50\mu\text{g DNA/ml})/(1 \text{ OD Unit}))$$

A second advantage of UV spectrophotometry is that it can be used to measure the purity of your DNA sample. Proteins have an absorption maximum of 280nm due to their tryptophan residues. Researchers routinely measure absorptions at 260 and 280nm and calculate the ratio of  $(\text{OD}_{260}/\text{OD}_{280})$ , a measure of purity, which should be between 1.65 and 1.85.

Unfortunately, a major prohibitive effect of UV spectrophotometry is the cost of a UV spectrophotometer and as a result simpler, UV independent assays have been developed by biotechnology companies.

NUCLEIC *dot*METRIC™ is a patented nucleic assay that provides quick and accurate measurements of DNA, RNA and oligonucleotides, without the need for a UV spectrophotometer.

NUCLEIC *dot*METRIC™ utilizes the principle of diffusion on a nylon membrane to determine the concentration of the nucleic acids. The concentration of nucleic acid can be measured either by measuring the diameter of diffused nucleic acid spots or comparing the color density of the nucleic acid spots with that of a set of known standards.

## TEACHER'S PRE EXPERIMENT SET UP

This kit is designed to quantitate DNA isolated with the Isolate Your Own Genome (BE-303), Polymerase Chain Reaction (BE-305), Purification of Gene (BE-306), Plasmid Isolation (BE-310, BE-311) and the Genomic DNA Isolation (BE-316, BE-317) kits, however samples in the range of  $10\mu\text{g}/\mu$  to  $1\text{ng}/\mu\text{l}$  can be measured accurately regardless of the isolation method or storage buffer.

For improved accuracy, we strongly recommend that the *dot*METRIC™ Application Device (Cat. # D217) and Application Capillaries (Cat. # A113) (sold separately) are used. The diluted DNA samples can be applied to the NUCLEIC Test Strips with a simple pipette and tip, however accurately pipetting  $1\mu\text{l}$  suffers from reproducibility and deviations of  $\pm 20\%$  can occur. If the *dot*METRIC™ Application Device is not used then use pipette tips with an outside bore diameter of 0.6-0.7mm, referred to as Application Tips in the protocol.

1. For measuring RNA or oligos, dilute the Nucleic Acid Dilution Buffer 100-fold. Mix  $5\mu\text{l}$  Nucleic Acid Dilution Buffer with  $495\mu\text{l}$  water.

## MATERIALS FOR EACH GROUP

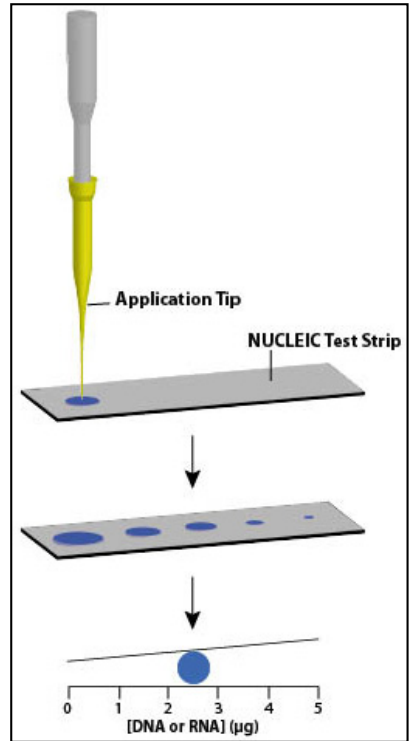
Supply each group with the following components.

- Nucleic Test Strips (50)
- 1 bottle Nucleic Dye (Shared with class)
- 1 bottle Nucleic Acid Dilution Buffer (Shared with class)
- 1 *dot*METRIC™ Scale (Shared with class)
- 1 *dot*METRIC™ Standard (Shared with class)
- 1 Application Board (Shared with class)
- Application Tips
- DNA Samples
- 1 Pair of Forceps
- Centrifuge Tubes

## PROCEDURE

### 1. Contact Capillary Method

1. Label two Centrifuge Tubes with your name and 1:4 and 1:10.
2. Pipette 15 $\mu$ l Nucleic Acid Dilution Buffer into the 1:4 tube and 45 $\mu$ l Nucleic Acid Dilution Buffer into the 1:10 tube.
3. Add 5 $\mu$ l DNA sample to each tube and pipette up and down to mix.  
*NOTE: For quantification of genomic DNA, rapidly pipette up and down 10 times to shear the DNA.*
4. Using the forceps, remove a NUCLEIC Test Strip from the box with the supplied forceps and carefully peel away the protective sheets.
5. Attach the NUCLEIC Test Strip to the application board with the side magnets and write your initials on one end with a soft pencil or ballpoint pen.  
*NOTE: Either side of the strip can be used.*
6. If using the dotMETRIC™ Application Device go to section 2, if not continue to step 7.
7. Apply an Application Tip to a pipette and pipette up 1 $\mu$ l of your 1:4 diluted sample.
8. Keeping the pipette tip completely vertical, as in figure, allow capillary action of the strip to draw the sample from the pipette tip. Do not use the pipette plunger to eject the sample.
9. Repeat the application procedure with the 1:10 DNA sample. Up to six samples can be loaded on a single NUCLEIC Test Strip.
10. Go to Section 3.



## **2. dotMETRIC™ Application Device Method**

1. Place the application board into the dotMETRIC™ Application Device. Place the clear plastic Application Capillary holder over the NUCLEIC Test Strip, by inserting into the grooves. Ensure the NUCLEIC Test Strip is directly under the holder.
2. Using the forceps to handle the 1µl Application Capillary, place the Application Capillary into the 1:4 diluted DNA solution. Watch the liquid rise to the top of the Application Capillary.
3. Position the Application Capillary in to the holes of the Application Capillary holder. Lower the capillary 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 Capillary is empty of solution, remove the Application Capillary from the device. The same capillary tip can be used for several applications.  
*NOTE: For applying more than a 1µl, apply the sample multiple times.*
5. Repeat steps 2-4 for the 1:10 diluted sample.
6. Go to Section 3.

## **3. Development of NUCLEIC Test Strips**

1. Once all samples are place the NUCLEIC strip in to a suitable developing tray and pipette 0.5ml NUCLEIC Dye directly onto the strip. Incubate at room temperature for 30 -60 seconds. Do not shake, move or agitate the tray as the dye will fall off the strip.
2. Pour off the NUCLEIC Dye and add 50ml pure water to the tray. Shake the tray for 10 seconds and discard the water. Add a second 50ml wash and shake until the blue background disappears (approximately 30-60 seconds).
3. For the NUCLEIC Test Strip prepared in Section 1, compare the size and color intensity to the NUCLEIC Standard reference included with the kit.
4. For the NUCLEIC Test Strip prepared in Section 2, use the dotMETRIC™ Scale to measure the size of the spot and determine the amount of DNA. If the spot is not spherical, use the dotMETRIC™ Scale to measure the widest and narrowest part of the spot and average the two concentrations.

5. Use the following calculations to determine the amount of DNA.

Test Sample Concentration = (Dilution Factor) x ((Scale Reading)/ (Amount Loaded (1μl))

For 1:4 dilution, dilution factor is 4 and 1:10 dilution factor is 10.

Example: 1:4 dilution gave a scale reading of 1.3μg from 1μl loaded.

$$= 4 \times (1.3/1) = 5.2\mu\text{g}/\mu\text{l}$$

1:10 dilution gave a scale reading of 0.6μg from 1μl loaded.

$$= 10 \times (0.6/1) = 6\mu\text{g}/\mu\text{l}$$

$$\text{Average} = (5.2 + 6)/2 = 5.6\mu\text{g}/\mu\text{l}$$

6. The NUCLEIC Test Strip can be dried at room temperature and kept as a permanent record.



## RESULTS, ANALYSIS & ASSESSMENT

Show your calculations and the DNA concentrations below:

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**RNA concentration (μg/ml) = (OD<sub>260</sub>) x (Dilution Factor) x ((40μg RNA/ml)/(1 OD Unit))**

**DNA concentration (μg/ml) = (OD<sub>260</sub>) x (Dilution Factor) x ((50μg DNA/ml)/(1 OD Unit))**

A second advantage of UV spectrophotometry is that it can be used to measure the purity of your DNA sample. Proteins have an absorption maximum of 280nm due to their tryptophan residues. Researchers routinely measure absorptions at 260 and 280nm and calculate the ratio of (OD<sub>260</sub>/OD<sub>280</sub>), a measure of purity, which should be between 1.65 and 1.85.

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## MATERIALS FOR EACH GROUP

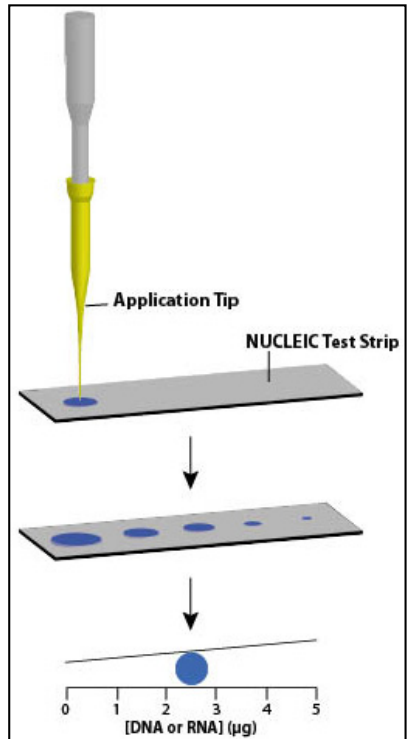
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- 1 Application Board (Shared with class)
- Application Tips
- DNA Samples
- 1 Pair of Forceps
- Centrifuge Tubes

## PROCEDURE

### 1. Contact Capillary Method

1. Label two Centrifuge Tubes with your name and 1:4 and 1:10.
2. Pipette 15µl Nucleic Acid Dilution Buffer into the 1:4 tube and 45µl Nucleic Acid Dilution Buffer into the 1:10 tube.
3. Add 5µl DNA sample to each tube and pipette up and down to mix.  
*NOTE: For quantification of genomic DNA, rapidly pipette up and down 10 times to shear the DNA.*
4. Using the forceps, remove a NUCLEIC Test Strip from the box with the supplied forceps and carefully peel away the protective sheets.
5. Attach the NUCLEIC Test Strip to the application board with the side magnets and write your initials on one end with a soft pencil or ballpoint pen.  
*NOTE: Either side of the strip can be used.*



6. If using the dotMETRIC™ Application Device go to section 2, if not continue to step 7.



7. Apply an Application Tip to a pipette and pipette up 1µl of your 1:4 diluted sample.
8. Keeping the pipette tip completely vertical, as in figure, allow capillary action of the strip to draw the sample from the pipette tip. Do not use the pipette plunger to eject the sample.
9. Repeat the application procedure with the 1:10 DNA sample. Up to six samples can be loaded on a single NUCLEIC Test Strip.
10. Go to Section 3.

## **2. dotMETRIC™ Application Device Method**

1. Place the application board into the dotMETRIC™ Application Device. Place the clear plastic Application Capillary holder over the NUCLEIC Test Strip, by inserting into the grooves. Ensure the NUCLEIC Test Strip is directly under the holder.
2. Using the forceps to handle the 1µl Application Capillary, place the Application Capillary into the 1:4 diluted DNA solution. Watch the liquid rise to the top of the Application Capillary.
3. Position the Application Capillary in to the holes of the Application Capillary holder. Lower the capillary 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 Capillary is empty of solution, remove the Application Capillary from the device. The same capillary tip can be used for several applications.  
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3. For the NUCLEIC Test Strip prepared in Section 1, compare the size and color intensity to the NUCLEIC Standard reference included with the kit.
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1:10 dilution gave a scale reading of 0.6µg from 1µl loaded.

$$= 10 \times (0.6/1) \quad = 6\mu\text{g}/\mu\text{l}$$

$$\text{Average} = (5.2 + 6)/2 \quad = 5.6\mu\text{g}/\mu\text{l}$$

6. The NUCLEIC Test Strip can be dried at room temperature and kept as a permanent record.

**RESULTS, ANALYSIS & ASSESSMENT**

Show your calculations and the DNA concentrations below:

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