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A Geno Technology, Inc. (USA) brand name

Protein dotMETRIC™

1µl Protein Assay

US Patent # 6174729

(Cat. # 786-20, 786-21)

INTRODUCTION

A proprietary test strip and reagent solutions have been developed such that when a 1-5 µl protein solution is applied to the test strip, it produces compact and symmetrical spots; the diameter of protein spots are directly proportional to their protein concentration (Figure 1). Thus, by measuring the diameter of protein spots with the dotMETRIC™ scale, supplied with each kit, you can reliably determine protein concentration.

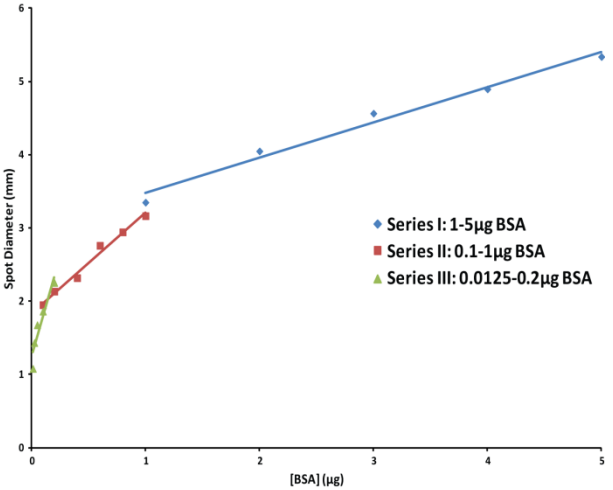


***Figure 1**: Plots show the linear relationship of protein concentration (BSA) with the protein spot diameters. The protein spot measuring scale, the dotMETRIC™ has been prepared using these plots. The dotMETRIC™ scale can be used to measure protein spots produced on the test strip and directly read the concentration of protein. The reliability of the dotMETRIC™ assay is substantially improved by making multiple dilutions of the sample solution each diluted solution then spotted onto the test strip. Averaging the readings from 2 or more protein spots yields a highly reliable (±5%) determination of protein concentration.*

NO PROTEIN-TO-PROTEIN VARIATION

Gelatin, BSA, Avidin, Alcohol dehydrogenase (yeast) and Thyroglobulin have been used to measure diameters of protein spots on the test strip at predetermined concentrations. It has been found that the diameters of protein spots on the test strip are not dependent on the nature and the origin of protein. Since the spot formation is not dependent on the amino acid composition of protein, the dotMETRIC™ assay is independent of protein-to-protein variation.

RESISTANT TO MOST COMMON LABORATORY AGENTS

The dotMETRIC™ assay is able to resist common laboratory agents such as Triton® X-100, Triton® X-114, Thesit, Tween® 20, Nonidet® P-40 and SDS, reducing agents such as DTT and β-mercaptoethanol, sugars, cobalt, EDTA, Tris buffers, and so forth.

DLD™ PROTOCOL

When protein concentration falls to a level where the diameters of protein spots are no longer large enough to be measured with the dotMETRIC™ scale, the assay kit uses an entirely new strategy for determination of protein concentration, called the Dilution to the Limit of Detection, or the DLD™ Protocol.

According to the DLD™ Protocol, when a protein solution is serially diluted and spotted onto the test strip, eventually a dilution is reached beyond which the protein spots are not visible; i.e., the dilution has reached the limit of detection (DLD™).

The dilution factor required to reach the limit of detection is used for the determination of protein concentration (**Figure 2**).

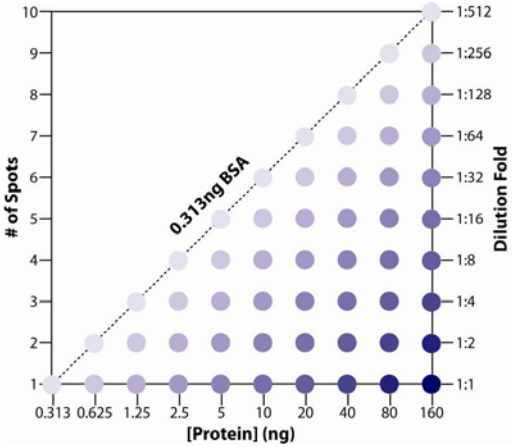


Figure 2: *An example of the Dilution to the Limit of Detection or DLD™ Protocol.*

The following proteins were used to determine the limit of detection for various proteins in the DLD-Protocol.

Protein	**DLD Concentration (ng)**
BSA	2.00
Avidin	1.7
Thyroglobulin	2.5
Alcohol Dehydrogenase (Yeast)	1.6
Carbonic anhydrate	2.6

A reliable estimation of protein can be made by multiplying the dilution fold of the last visible spot by (2.0± 0.1) ng.

Since the DLD™ factor of protein is dependent on amino acid composition; the DLD™ protocol shows protein-to-protein variation. Therefore, the DLD™ protocol is a highly useful and sensitive method if you have established the DLD™ factor for your protein. In the absence of an established DLD™ factor, like other widely used colorimetric methods, the DLD™ Protocol is subject to protein-to-protein variation.

ITEM(S) SUPPLIED

Description	Cat. # 786-20	Cat. # 786-21
Fixer A	60ml	60ml
Developer B	2 x 60 ml	2 x 60ml
Dilution Buffer	15ml	15ml
Test Strips	50	50
dotMETRIC™ Scale	1	1
Spot Application Board	1	1
Plastic Forceps	1 Pair	1 Pair
dotMETRIC™ Application Device	-	1
1µl Application Capillaries	-	100

ADDITIONAL ITEM(S) REQUIRED

The following items are supplied separately for use with dotMETRIC™ assay:

- Pipette Application Tips (96) -0.7mm, Compatible for assay use. (Cat. # 786-64)
- 1µl Application Glass Capillary tips. (Cat. # 786-23)
- Developing Trays (Set of two) (Cat. # 786-24)
- dotMETRIC™ Spot Application Device (Cat. # 786-63)

NOTE: *For ordering information, visit www.GBiosciences.com*

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Store the kit in its original box at room temperature upon arrival.

WARNING*: Storage in cold is detrimental to the kit reagents*

PROTOCOLS

There are three different methods for using dotMETRIC™ for protein concentration determination, select the appropriate protocol from below:

QUICK PROTOCOL: dotMETRIC™ OVERVIEW

Please read the detailed protocol and information before attempting to use the dotMETRIC™ protein assay.

Step 1: Protein Dilution

Dilute 1µl of test protein sample with 5 or 10µl of Dilution Buffer.
(See Step 1 of dotMETRIC™ Protocol 1)

Step 2

Using forceps; remove a test strip from its protective cover and mount on the application board.

Step 3

Apply 1-5 µl of the diluted protein solution on the test strip.

Step 4: Develop the Test Strip

A) Prepare Fixer A: Mix 0.8ml Fixer with 7ml deionized water in an incubation tray. Place test strip in diluted Fixer A. Incubate at room temperature for 2 min.
B) Prepare Developer B: Mix 1.6ml Developer with 6.2ml deionized water in an incubation tray. Transfer the test strip to the diluted Developer B. Gently shake the tray for 30 seconds. Incubate 2-4 minutes or longer at room temperature. Shaking is not recommended during incubation. Spots become visible within 2-3 minutes.

Step 5: Determine Protein Concentration

Use the dotMETRIC™ scale to match the diameter of each spot to its corresponding protein concentration.
(See How to Use the dotMETRIC™ Scale)

dotMETRIC™ PROTOCOL 1: USING A PIPETTE

G-Biosciences recommends the dotMETRIC™ Spot Application Device (Cat. # 786-63) that improves the accuracy of the assay and saves time in performing the assay. The dotMETRIC™ assay can be used without the Spot Application Device, however extreme care must be taken when applying spots to the test strip. For accurate spot application with a pipette tip follow these guidelines:

- Use an ultra fine pipette tip. Outside diameter at tip end of less than 0.7mm. Suitable pipette tips are our Pipette Application Tips (Cat. # 786-64)
- Avoid movement once in contact with the strip, this will lead to variation in protein concentration.
- Do not eject the solution from the tip, protein solutions are applied by capillary action.
- For multiple spots, space 7-8mm and apply 8-9 spots/ strip.

1. Take 1µl of test protein sample and mix with 5-10 µl (or higher volume) of Protein Dilution Buffer. Protein samples must be mixed with Protein Dilution Buffer for the correct performance of the dotMETRIC™ assay.
   a. *For the majority of assays, mix 1µl protein sample with 5µl Dilution Buffer.*
   b. *For protein solutions with high concentration of the following agents, mix 1µl protein solution with 10-20 µl of Dilution Buffer*
      - *>60mM Tris*
      - *>50mM salt*
      - *>2% detergent*
      - *>10% ammonium sulfate*
      - *8M urea*
      - *Excessive concentration of other agents.*
   c. *For samples with a high protein concentration, dilute with an appropriate volume of Dilution Buffer to produce spots with <2µg protein per spot.*
2. Position an appropriate 1-10µl micropipette tip on the pipette and withdraw 1-5µl of protein solution. Ensure there is no air bubble trapped in or drop hanging from the tip. Remove the tip from the solution by touching the wall of the container.
3. Carefully position the pipette tip containing 1-5µl of protein solution vertically on the dotMETRIC™ Test Strip. Allow the protein solution to slowly diffuse out of the tip into the Test Strip by capillary action. Do not push the pipette plunger or force the solution out of the tip. Allow the protein solution to diffuse on its own into the Test Strip; this normally takes 20-100 seconds (for 1-5µl solution). Do not move the tip while the protein solution is diffusing into the Test Strip.
4. Make 2 spots for each sample. Apply 2µl and 4µl on the Test Strip.
   NOTE: *You may choose to make only one spot for each sample or apply 1-5µl on each spot.*

5. Develop the Test Strips by mixing 0.8ml Fixer and 7ml DI water in a suitable incubation tray. Place Test Strip in diluted Fixer A. Incubate at room temperature for 2 min.
6. Prepare Developer B by mixing 1.6ml Developer and 6.2ml DI water in an incubation tray. Transfer the Test Strip to the diluted Developer B. Gently shake the tray for 30 seconds. Incubate 2-4 minutes or longer at room temperature. Shaking is not recommended during incubation. Spots become visible within 2-3 minutes.
7. **Determine Protein Concentration**: Use the dotMETRIC™ scale to match the diameter of each spot to its corresponding protein concentration. See "How to Use the dotMETRIC™ Scale".

dotMETRIC™ PROTOCOL 2: USING SPOT APPLICATION DEVICE

The G-Biosciences dotMETRIC™ Spot Application Device (Cat. # 786-63) greatly improves the accuracy of the assay and saves time in performing multiple assays. The dotMETRIC™ Spot Application Device should be used in a well lit that allows researchers to see the movement of the protein solutions to and from the 1µl capillary tubes.

1. Take 1µl of test protein sample and mix with 5-10 µl (or higher volume) of Protein Dilution Buffer. Protein samples must be mixed with Protein Dilution Buffer for the correct performance of the *dot*METRIC™ assay.
   a. *For the majority of assays, mix 1µl protein sample with 5µl Dilution Buffer.*
   b. *For protein solutions with high concentration of the following agents, mix 1µl protein solution with 10-20 µl of Dilution Buffer*
      - *>60mM Tris*
      - *>50mM salt*
      - *>2% detergent*
      - *>10% ammonium sulfate*
      - *8M urea*
      - *Excessive concentration of other agents.*
   c. *For samples with a high protein concentration, dilute with an appropriate volume of Dilution Buffer to produce spots with <2µg protein per spot.*
2. On the dotMETRIC™ Spot Application Device, remove the clear plastic Application Capillary holder and the two magnetic dotMETRIC™ Test Strip holders. Wearing gloves and using the supplied forceps position the dotMETRIC™ Test Strip in the center of the Application Board and secure with the two magnetic dotMETRIC™ strip holders. Position the clear plastic Application Capillary holder over the dotMETRIC™ Test Strip. Ensure the holes in the holder are centered over the Test Strip, if not reposition the Test Strip.
3. Using the supplied forceps, place the 1µl Application Capillary into the diluted protein solution from Step 1. Allow the protein solution to move to the top of the Application Capillary, this will take ~5 seconds.
4. Position the full Application Capillary in the holes provided in the device and lower the capillary tip until the tip touches the Test Strip membrane surface. As soon as the tip touches the membrane, the solution will begin to flow in to the membrane.
5. After the samples have been diffused into the membrane and the application tip is empty of the solution, remove the Application Capillary from the device.
   NOTE: *The same application tip may be used for several applications.*
6. Make 2 spots for each sample. Apply 2µl and 4µl on the test strip. For applying >1µl for each spot, reapply the sample with the same Application Capillary to the spot. The spot does not need to dry between each application.
   NOTE: *You may choose to make only one spot for each sample or apply 1-5µl on each spot.*

7. Develop the Test Strips by mixing 0.8ml Fixer and 7ml DI water in a suitable incubation tray. Place test strip in diluted Fixer A. Incubate at room temperature for 2 min.
8. Prepare Developer B by mixing 1.6ml Developer and 6.2ml DI water in an incubation tray. Transfer the test strip to the diluted Developer B. Gently shake the tray for 30 seconds. Incubate 2-4 minutes or longer at room temperature. Shaking is not recommended during incubation. Spots become visible within 2-3 minutes.
9. **Determine Protein Concentration**: Use the dotMETRIC™ scale to match the diameter of each spot to its corresponding protein concentration. See "How to Use the dotMETRIC™ Scale".
10. Take 1µl of test protein sample and mix with 5-10µl (or higher volume) of Protein Dilution Buffer in a 0.5ml micro centrifuge tube. See Protein Dilution Guide for more information.

dotMETRIC™ PROTOCOL 3: DLD™ PROTOCOL

When protein concentration falls to a level where the diameters of protein spots are no longer large enough to be measured with the dotMETRIC™ scale, the assay kit uses an entirely new strategy for determination of protein concentration, called the Dilution to the Limit of Detection, or the DLD™ Protocol.

1. For the DLD™ protocol, prepare two serial dilutions in dilution buffer.
   a. Serial Dilution I: Prepare 5 dilutions in which each successive dilution is 2-fold, a 1:1 mix with Protein Dilution Buffer. This gives a 2X, 4X, 8X, 16X and 32X dilution.
   b. Serial Dilution II: Prepare 4 dilutions in which the first dilution is 3-fold (1:2 mix with Protein Dilution Buffer) and each successive dilution is 2-fold. This gives a 3X, 6X, 12X, and 24X dilution.
2. Spot 1µl from each dilution onto the Test Strip by following step 2 in either the B) Using a pipette dotMETRIC™ Protocol, or C) Using Spot Application Device dotMETRIC™ Protocol.
3. Following development of the Test Strip(s), find the limit of detection or the last visible spot at the highest dilution fold. Multiply the dilution fold of the last visible spot by the DLD™ factor 2±0.1ng, the DLD™ factor of BSA.
   NOTE: *For routine protein estimation of a known protein in your laboratory, it is recommended that you use a known concentration of your protein to first establish the DLD™ factor for your protein. Once you establish the DLD™ factor for your protein, it will enable you to determine a highly reliable concentration for your test samples. Simply multiply the DLD™ factor with the dilution fold of the last visible protein spot.*

HOW TO USE THE dotMETRIC™ SCALE

1. It is important the developed Test Strip is read before it is completely dry and within 10 minutes of removing from the Developer Solution.
2. Readings should be made under bright light. Position the protein spots between the two lines of the dotMETRIC™ such that the outer margins of the spots touch the underside of the lines, as shown in Figure 3. Read the protein concentration from the horizontal scale, where the spot first touches the scale (the center of the spot).

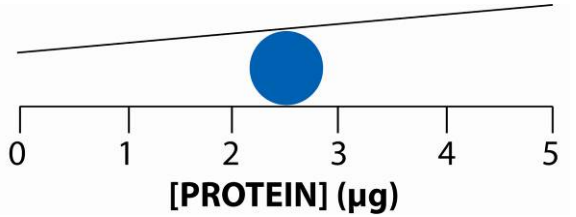


***Figure 3**: Position the spot between the two lines (Image not too scale).*

3. Read each spot twice, reading corresponding concentration for a long and a short diameter of each spot, and calculate a mean average concentration for each spot (Figure 4).

   ***NOTE:** It is normal for some spots to have a darker region and a light color fuzzy "halo" around them. Measure the larger spot, including the halo. If you only measure the darker region of the spot the reading would be reduced approximately by 15%. Adjust the reading by multiplying with a factor x 1.175. When you make a photocopy of the test strip, depending on the darkness setting of the photocopier, you might not record the halo.*

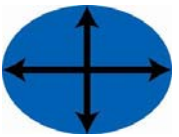


Figure 4

4. Multiply the readings of each spot (µg protein/spot in volume applied) by the corresponding dilution fold.

   Protein Concentration = Dilution Fold x [µg protein in spot / µl volume applied]

EXAMPLE: 1µl test sample is diluted with 5µl Dilution Buffer, the sample is diluted 6x fold (Dilution Fold = 6). 2µl of the diluted sample is applied on the test strip that reads a protein concentration of 0.6µg.

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

***NOTE:** If you examine the readings of individual spots in a test, you will notice large deviations (up to ±5-20%). Such deviations are normal and expected. When you calculate a mean average from 2-3 spots, the standard deviation will drop to ±5%. It is, therefore, recommended that you produce 3-4 spots from each sample and calculate a mean average from the best spots.*

MAKING A PERMANENT RECORD

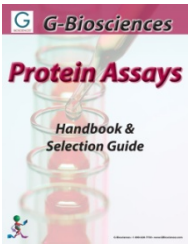
Test strips may be stored refrigerated in developer for several weeks without loss of color. After drying the test strips, protein spots remain visible for several hours and then begin to fade with time. Protein spots can be regenerated by redeveloping the test strip. To make a permanent record, mount the test strip on a white sheet of paper and make a photocopy or scan at normal setting.

TROUBLESHOOTING

- If you notice a large deviation between samples of identical concentration, check your pipetting technique. Accurately pipetting 1µl requires skill and caution. A deviation of up to ±20% could be due simply to pipetting errors. Alternatively, use Spot Application device.
- The protein solution can precipitate when mixed with dilution buffer. This might happen if the protein solution is not mixed immediately and vigorously with the dilution buffer.
- If the protein spots are consistently smaller or larger than the expected value, check the calibration of your pipette. Your pipette may require re-calibration. Alternatively, use Spot Application device
- Spots are too large or if all spots are same size. Check your spotting technique. Make sure that you are diluting protein solution with the dilution buffer provided and allow the protein solution to slowly diffuse out of the tip into the test strip. Do not force solution out of tip.
- No spot develops. Redevelop the test strip. If you still do not see protein spots then either you do not have enough protein in your sample or the reagents have expired.
- Spots are faint. Some proteins develop faint spots. It will not affect your results. You may try to redevelop the test strip and incubate in cold and dark to enhance darker spots.
- Spots have a halo around them. It is normal. Measure the larger spots, including halo. Use Spot Application device. Multiple applications on the same spot reduces halo formation.
- Uneven spots. Improve your spotting technique. Make sure the strips are not soiled. Measure the long and short diameter of the spots and calculate mean average.
- Spots give lower than expected results. Make a higher dilution with dilution buffer, 10-20 folds or even higher. See “Protein Dilution Guide”.

RELATED PRODUCTS

Download our Protein Assays Handbook.



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