415PR-02

Protn-Latex™

For Determination of Protein in Latex

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

INTRODUCTION

Protn-Latex[™] is a simple and reliable method for estimation of protein contamination in latex. Protein estimation can be performed either on the production site or in any laboratory with a minimum of skill or instrumentation. The test detects as low as 25-40μg protein /gm latex (or 25-50 parts per million).

PRINCIPLE

When a sample of protein solution is applied on the test strip it produces compact and symmetrical protein spots, the diameter of protein spots are proportional to protein concentration (Fig. 1). The test kit is supplied with latex protein extraction buffer. After extraction of protein from the latex sample, the extract is applied on the test strip and the test strip is developed to produce protein spots. By measuring the diameter of protein spots with the **Protn-Latex**[™] card (supplied with each kit) you can reliably determine the range of protein concentration in the sample. For more information read – Alam. A, *Rubber World*: 213, No. 4, Jan. 1996, p.17.

ITEM(S) SUPPLIED

Description	Cat. # 786-20Latex	Cat. # 786-21Latex
Test Strips	50	50
Developer-I	50ml	50ml
Developer-II	50 tubes	50 tubes
Latex Protein Extraction Buffer	25ml	25ml
Application Board	-	1
Capillary tips (5μl)	-	1
Droppers	5	5
Protn-Latex[™] Card / dotMETRIC[™] Scale *(Small)*	1	1
Forceps	1 pair	1 pair

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Store all kit components at room temperature.
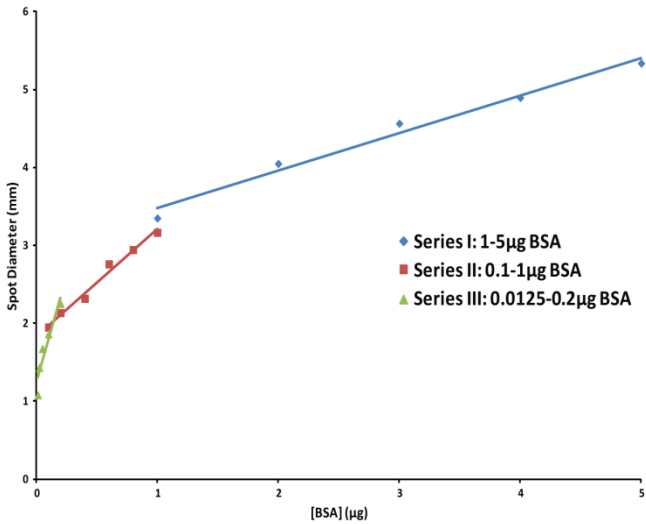
Figure 1: Shows linear relationship of protein concentration (Bovine Serum Albumin, BSA) with the diameters of protein spots. A protein spot measuring scale, the Protn-Latex™ card, has been prepared using these plots for measurement of protein concentration in test samples.

ADDITIONAL ITEMS REQUIRED

- Spot Application Device (One time purchase, Cat # 786-63)
- Tubes with matching Pestles
- Micro tubes
- Centrifuge, and
- Developing Trays.

DETERMINATION OF PROTEIN CONCENTRATION

Determination of protein in test samples involves the following three steps:

A. Extraction of protein from the test samples.
B. The extracted protein is applied on the test strip. The test strip is developed to produce protein spots.
C. Protein spots on the test strip are measured to determine range of protein concentration.

A. EXTRACTION OF PROTEIN FROM LATEX SAMPLES

| **1" x 1"** | **½" x ½"** | |
| **25mm x 25mm** | **12mm x 12mm** | **10mm x 10mm** |
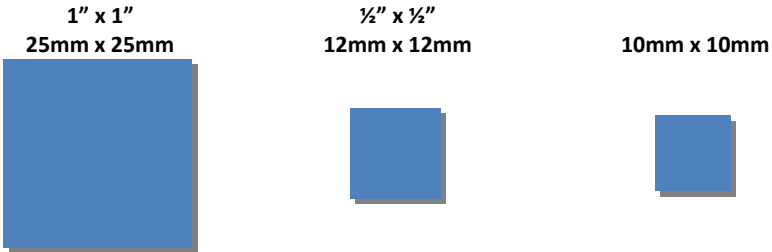
1. Take a small piece of the latex sample to be tested. For liquid samples (serum or sap), read the instructions below. Weigh the sample for determination of protein concentration per gram weight of the latex. For convenient handling, the total weight of the sample should be 0.1-0.2 grams per sample. Make note of the sample size and weight.

2. Shred the latex into small pieces (use scissors or a blade for shredding) and transfer the pieces to a micro test tube. Label the tube.

3. Using the dropper provided with the test kit, add a few drops of the Extraction Buffer to the latex sample. Make note of the number of drops (volume) added into the sample.
   NOTE: 1 Drop ~ 0.022ml. You may also use a high calibration pipette, and record the volume added into the sample.

4. Agitate or vortex the sample periodically (20-30 minutes) to facilitate extraction of protein from latex sample.
   NOTE: Read the note on Extraction Method.

5. After extraction is complete, leave the tube standing for 1-2 minutes. This will allow the extraction buffer (containing extracted protein) to settle to the bottom of the tube.

6. Prepare the sample application device for sample application. Using the forceps provided, remove a Test Strip from the container. Remove and discard the red marked protective covering. Position the test strip in the central lane of the application board and secure the test strip with two magnetic strips. Re-position the central bar with 6 spot application positions.

B. APPLICATION OF SAMPLE ON THE TEST STRIP

1. Use a 5µl capillary tip provided with the kit. Drop the capillary tip into the tube. Allow the capillary to reach the bottom of the tube (into the extraction buffer accumulated in the bottom of the tube). Hold the tube between the fingers and tilt the tube to horizontal position and allow the extraction buffer to rise and fill the capillary tube (takes 1-2 seconds to fill the capillary). While holding the tube horizontally, use the forceps and remove the capillary. Position the capillary into one of the application positions of the application device to stand vertically on top of the Test Strip.

2. The extraction buffer will begin to diffuse into the test strip. It will take 1-2 minutes for the entire sample to diffuse into the test strip. Preferably, apply 2 spots for each sample.
   NOTE: You may apply up to 6 samples on each strip.

3. After the sample application is complete, remove and discard the capillary tips. You are now ready for developing the test strip.

4. Remove the test strip from the sample application tray and place in a developing tray. Using a clean dropper, apply 10-20 drops of the Developer-I on top of the Test Strip to cover the entire Test Strip. (Immediately replace the cap of the Developer-I bottle).

5. Make sure the test strip is fully covered and wet with the Developer-I. Incubate for 2 min.

6. Remove the test strip from the developer. Shake the test strip to remove excess Developer-I
   NOTE: Excess Developer-I on the test strip will turn Developer-II darker and difficult to examine.

7. Introduce the Test Strip into the Developer-II tube. Close the cap and invert the tube 5-6 times. Allow the tube to stand at room temperature for 5-10 minutes. A blue color protein spot will develop in the sample containing protein.

C. MEASURING THE PROTEIN SPOT FOR CONCENTRATION

1. Measure the protein spot developed on the test strip to determine protein concentration in the test sample. Read the application note on "How to measure a protein spot for protein concentration?"

2. Scan the Test Strip for permanent record. Test strips can also be stored and redeveloped.

3. Use the work sheet to calculate and report protein contamination in test samples.

INSTRUCTIONS FOR USING LIQUID SAMPLES (e.g. SERUM)

1. Use a clean dropper (or other device) and transfer one drop (0.01ml) into a micro-tube. Add 20 drops (0.2ml) of Latex Protein Extraction Buffer. Mix the contents of the tube thoroughly.
2. Leave the tube standing for 5-6 minutes. This will allow the extraction buffer (containing extracted protein) and serum to separate into two separate layers.
   NOTE: In some samples, the mixture might not separate into separate layers, in such cases centrifuge the mixture for 5 minutes in a bench top micro-centrifuge.
3. Use a clean dropper (or other device) and remove one drop of the extract from the tube and transfer into a clean micro-tube.
4. The extract in now ready for protein measurement. Follow the protocol sections B & C.

APPLICATION NOTES

1. Methods of Protein Extraction from Latex Samples

Shred the latex into small pieces and transfer into a micro tube. Use the Extraction Buffer to extract protein from the latex sample. Add a few drops of the extraction buffer to simply wet the sample pieces. Do not add larger volume of the extraction buffer, this will dilute the extracted protein and lower the detect ability (sensitivity).

Allow latex pieces and Extraction buffer to mix thoroughly. Mixing can be achieved by gently rotating the tube, either in hand or in a rotating device. Rolling the sample between hands is one simple way to mix Extraction Buffer and the latex pieces. Do not shake the tube it will create froth.

Extraction Buffer contains agents that will extract most protein from latex within 20-30 minutes; however, some applications may require longer extraction. Run tests to establish appropriate extraction time for your sample. The Extraction can be greatly facilitated by grinding the latex sample pieces with a pestle (see accessories) in the presence of a minimum volume of the extraction buffer.

After extraction process, the extraction buffer can be separated from the pieces of latex by centrifugation or simply allowing the test tube to stand vertically for 2-3 minutes.

Powdered Latex: If the latex product is powdered then it is important to remove powder from the samples.

To remove the powder material before extraction, simply shake the sample in pure water for 2-3 seconds. Dry the latex sample by pressing between paper towels and then start extraction procedure.

Alternatively, after extraction procedure, centrifuge the extract for 3-5 minutes and collect the clear supernatant.

2. Using the Protn-Latex™ Card to Measure Protein

The protein spots are generally symmetrical. Occasionally, the protein spots may appear irregular; in such cases consider the larger diameter of the spot. In some cases the spots may have a lighter center surrounded by a halo around them. It is normal. Measure the large spots, including halo.

The protein spots may simply be compared with the set of standard spots shown on the *Protn-Latex*™ card to determine the range of protein concentration. The amount of protein in a spot may be represented as higher or lower than a standard protein spot on the *Protn-Latex*™ card.

Alternatively, the spots may be measured with the sliding scale printed on the card. Position the protein spots between the two lines of the *Protn-Latex*™ card such that the outer margins of the spots touch the underside of the lines, as shown in Figure 2. Read protein concentration off the horizontal line. The concentration you read will be µg of protein (per µl applied) on the Test Strip.
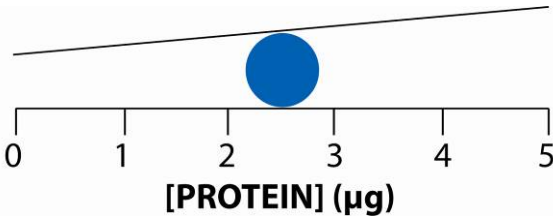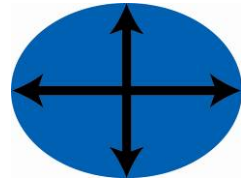

Figure 2	Figure 3

Read each spot twice, a long and a short diameter of each spot and calculate a mean average diameter for each spot (Fig 6). For improving reliability of the measurement, apply 2-3 spots from each sample and calculate a mean average.

CALCULATE TOTAL PROTEIN IN TEST SAMPLE

I. Measure the amount of protein in each spot (i.e. µg protein per 0.005ml sample applied on the spot)

II. Calculate total protein in the volume of the extraction buffer use for extracting the protein from the sample, as follows-

   [(µg estimated protein in spot) x (total volume of extraction buffer, ml)] /0.005ml

   NOTE: Total volume of Extraction Buffer Used = (# drop) x average volume of each drop (1 Drop ~ 0.022ml).

III. Calculate total protein per gram sample weight (or size of the sample) as follows-

   Total protein in sample / weight of the sample

The protein spots begin to fade around the edges as it dries. Read the test strip before it is completely dry. The fading protein spots may be regenerated by redeveloping the test strip (follow the sample procedure).

For convenient reading and permanent record of the test strips with the *Protn-Latex*™ Card we recommend making a photocopy of the test strip at dark setting (darker setting of photocopier will enhance recording of faint margins and even faint spots). When you are ready to photocopy the test strips, press the test strip between a tissue paper to remove excess developer and then quickly photocopy the test strip. Do not wipe the test strip.

3. Making Permanent Record

Test Strips can be stored in developer for several weeks (refrigerated) 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. For making a permanent record, mount the test strip on a piece of white paper and scan or photocopy at dark setting.

4. Material Safety Data & Disposal of Waste

The kit does not contain any toxic compounds and therefore does not required any extraordinary precaution. The kit contains diluted solution of acids. Observe general laboratory precaution. Avoid contact with eyes and skin or breathing over the reagent solution. In case of accident, immediately wash the contaminated area with plenty of water and contact your physician. Waste can be discarded through laboratory water sink.

5. In Liquid Sample - (Sap Or Serum)

Take a drop of the sample and transfer into a tube. Add 10 drops of the Extraction Buffer. Mix for 5 minutes. Leave the tube standing for 5 minutes.

6. Accessories:

The following items for use with *Protn-Latex*™ assay are supplied separately:
- Spot Application Device (one time purchase, Cat. # 786-63).
- Tubes & matching pestles, micro tubes, centrifuge, and developing trays.
- Protein standards can be used for spot generation and comparison to unknown samples. G-Biosciences recommends our BSA Protein Standard (Cat. # 786-006) or our Non-Animal Protein Standard (Cat. # 786-438).

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

Last saved: 4/4/2014 CMH

Sample ID #	Sample Size (mm x mm)	Sample Weight (mg)	Extraction Buffer Used (# drop) 1 Drop ~ 0.022ml # drop x 0.022ml	Extract Applied to test Strip (0.005ml)	Protein per spot (µg /spot)	Total Protein in Extract	Protein concentration per g sample	Protein concentration Part per million

Sample ID #	Position the Test Strip in the area provided below	Comment

Test Strip Scan/Photocopy Record

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