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1.0 Purpose

The purpose of this procedure is to analyze gels, blots, dot blots, etc. by means of densitometry using the Gel-Pro 6.0 Analyzer software and an image source.

2.0 Scope

Process Analytics/Quality Control (PA/QC) personnel will perform this procedure. Other Biopharmaceutical Development Program (BDP) personnel may use this protocol for development or in-process analysis.

3.0 Authority and Responsibility

3.1 The Director, PA/QC has the authority to define this procedure.

3.2 PA/QC is responsible for assignment of this procedure/training.

3.3 PA/QC personnel are responsible for performing this assay and following this procedure.

3.4 Biopharmaceutical Quality Assurance (BQA) is responsible for reviewing and approving this test.

3.5 BQA is responsible for quality oversight of this procedure.

4.0 Starting the Gel-Pro Analyzer and Opening an Experiment

4.1 To start the Gel-Pro analyzer system, and begin working with it, the operator must open the main window. Follow the steps below.

4.1.1 Turn on the computer.

4.1.2 Select Start: Programs.

4.1.3 Select Gel-Pro 6.0 Analyzer and double click on Gel-Pro Analyzer.

4.2 When first starting the Gel-Pro, the Experiment window appears in the center of the screen. Otherwise, to open an experiment, follow the steps below.

4.2.1 Click on the File menu.

4.2.2 Click on Experiment from the list of options. The Experiment dialog box will appear.

4.3 The title, experimenter, function type, and description areas will appear blank in the Experiment Window. Enter the BQC Test Request number for the title and type the operator's name for Experimenter.

4.4 Select "Protein Analysis" as the function type for Tris-Glycine gels, NuPAGE gels, and IEF gels. To see a list of available function types, click on the drop-down button in the Function Types area. Depending on the functions that have been selected, Gel-Pro will automatically set the defaults for that type of experiment. Click "OK" when information is complete.

5.0 Loading an Image

5.1 The first step in any process is to load an image into the workspace. Images can be loaded from the following sources: a scanner, video-capture device, or from a stored file.

5.2 To retrieve a stored image, click on the File menu and select Open Image. This option allows the operator to select from a list of image files stored on the operator's hard drive or diskette. The operator will see the Open File dialog box. To select a file, move the cursor to highlight the file name from the list box, and click on Open. The image that has been selected will be visible in the Gel-Pro workspace.

5.3 To acquire an image directly from the scanner, place the gel face down on the scanner, click on the Acquire menu, and select Scan. From the Epson Scan Task manager, select "Setting 1" from the drop-down list in the settings section, then click "Preview". The scanner will then scan a preview of the image. Left click and hold to drag a box around the desired image. Release the left click button on the mouse. If desired, the box can be tightened around the desired image. A crop option will be available later in this procedure so it is not necessary to have a perfectly cropped or perfectly straight image at this point. Click "Scan" to scan the image to Gel Pro 6.0.

6.0 Selecting Options from the ID-Gels Tool Palette

6.1 The ID-Gels tool palette appears on the right side of the screen after the operator has selected ID-Gels option from the menu bar. The options in the ID-Gels tool palette are the tools that will be used to perform the experiment.

6.2 Rotating an Image

6.2.1 Frequently, the first step will be to rotate the image. The picture must be oriented so that the lanes in the gel will be presented as vertically as possible. This is very important, as the program will attempt to find vertical lanes. Rotating the image allows the operator to correct any accidental skewing that may have occurred in the process of scanning the gel, a photograph of the gel, or x-ray film.

6.2.2 Click on the Rotate button from the ID-Gels tool palette.

6.2.3 Type in the angle of rotation required or use the mouse to move the grid.

6.2.4 Once the image has been straightened, click on the “OK” button in the Rotate dialog box.

6.2.5 To crop the image, click on the box-shaped icon in the main toolbar. Left click and drag a box around the image. Click “Edit”, scroll down and click “Duplicate/Crop”. The cropped image will appear in a new window. Close the original (un-cropped) image without saving it. The straightened, cropped image will be saved in the next step.

6.2.6 Click on the "OK" button in the Rotate dialog box. Click "File" and “Save”. Save the image using the BQC Test Request number and a suffix if there are multiple gels associated with the current test request. This step saves the color image which is used to create formal gel summaries included with reports or to be used in presentations. Save this image in the Gel Scans folder

6.3 Identifying Lanes

6.3.1 Select "Lanes" and then the Color Image ID-Gel Analysis screen appears. Select “Extract Red Channel," then “OK." This will change the gel’s image. The gel will now appear Black and White. If gels are Silver stained or a Western Blot is being analyzed, “Extract Green Channel” may be a better option. Save this image in the Gel-Pro “Images” folder.

NOTE: See Section 7.0 – Saving the Experiment. It is good practice to save the experiment frequently throughout the process of analysis. In the event of a power outage or a computer/network problem, analysis can resume from the most recent save point rather than having to start over completely.

6.3.2 To begin identifying the lanes in the image, select “Find Lanes” from the Lane dialog box. The Gel-Pro Analyzer will automatically identify the lanes in the image by superimposing colored outlines over the lanes. The Lanes and the Lane Profile dialog box will now have every option button available for use. From the Lanes

Dialog box, the operator can "Show Lanes," "Set the Lane Width" in pixels, "Add" or "Delete Lanes," "Find Lanes," "Label Lanes," and work with "Straight or Curved Lanes." There are also check boxes on the right side which allow the operator to set options such as, "Always Show Lanes," "Find Lanes when Dialog appears," "Force Straight Lanes," "Uniform Lane Width" or "Find Lane Width Automatically."

6.3.3 If a lane in the image has been overlooked, it can be added manually. Click on the “Add Lanes” button in the lanes dialog box. The operator will be instructed to move the cursor to the area where a straight line is needed and click the left mouse button, followed by "OK." If, however, lanes appear over wells that the operator does not want to analyze, remove the lanes by selecting “Delete lanes” and clicking on the lane to be deleted.

6.3.4 Lane width can be adjusted by selecting “Uniform Lane Width." This will allow the operator to type a value in the lane width area or by using the spin buttons to increase or decrease the value shown. Lane Width is given in pixels. By unchecking the “Uniform Lane Width” box, the lane width of a single lane can be adjusted.

6.3.5 Lane Length can be adjusted by moving the cursor over the top or bottom of a lane until an arrow appears. Increase or decrease the lane length by pulling the top of the box just above the highest band on the gel and pulling the bottom of the box just below the lowest band on the gel.

6.3.6 The “Curve Lanes” button allows the operator to adjust the lane to be certain that the bands are completely centered inside the lane box. Gel-Pro automatically superimposes red control points over the lanes in the image. Use the left mouse button to “pull” these control points to manipulate the curve of the lane. The default is five control points per lane. Click “OK” when finished curving the lanes. Gel-Pro will redraw the red outlines as curves over the lanes in the image. When working with an image in which the lanes are curved and there are protein bands consistently throughout the lane (example: Washed inclusion bodies or cell paste samples) the automatic lane curve feature can be used. Uncheck the “Force Straight Lanes” option on the “Lanes” dialog box. Click the “Find Lanes” button. Gel-Pro conforms the lane markers to the curve of lanes in the image. The lanes can be curved further manually by using the “Curve Lanes” function.

6.3.7 Click on the “Labels” Button to individually label the lanes. By default, the lanes are numbered sequentially from left to right, with the numbers appearing at the top of the lanes. To change a lane label, move the cursor over the number at the top of the lane the operator wishes to label. Type the name of the label and select whether the label is on the top or bottom of a lane. Click the green checkmark icon and move to the next lane that needs to be labeled. Gel-Pro automatically displays the new lane label in the specific location. When the operator has labeled the lanes with the proper number, click the “OK” button. This completes the options in the Lane Dialog Box. Click “OK” to move to the next section – Bands.

NOTE: Typically, the lanes are labeled according to the gel lane assignment sheet with the label placed at the top of the gel. The bands are Not typically labeled.

6.4 Identifying Bands

6.4.1 Select “Bands” in the ID-Gels tool palette, and the Band Dialog Box will appear. From this box the operator can "Add or Delete Bands," "Find Bands," "Curve Bands," "Label Bands," and Change various "Options." The Band Dialog box also allows the operator the option of “Dark Bands, Bright Background” or “Bright Bands, Dark Background." Typically “Dark Bands, Bright Background” is selected. This can be changed depending on the appearance of the gels image.

6.4.2 The operator begins by selecting “Find Bands." Bands, represented by a + symbol, will automatically appear over the bands in the image. If unwanted bands appear on the gel’s image, delete them manually by using the “Delete Bands” button. Put the cursor over the unwanted band (+) and click the left mouse button. The + symbol over the band will automatically disappear. Click “OK” when finished. If the automatic identification function overlooked a band, the operator can add a band marker by clicking the “Add Bands” button. The operator can also adjust the minimum band height (%) from the band dialog box. 1 is typically used, by changing the number to 0.5 more band will automatically appear when “Find Bands” is selected. Click on the band, and a + symbol will appear over the band. Click “OK” when finished.

6.4.3 Because bands are not always formed as straight horizontal lines in the gel, Gel-Pro allows the operator to curve band markers to conform to the curve of bands in the image. Select “Curve Bands," then “Add Curves." Left-click on the bands to be curved. Band markers for the selected bands are displayed as lines with five control points. Click and pull the control points to follow the contour of the curved bands. When complete, Click “Done." Gel-Pro automatically re-finds the bands in the lane after adding curves. Add missed bands or delete incorrectly “found” bands in the lane after curving bands.

6.4.4 "Label Bands" allows the operator to change the band label. By default, the bands are given a system row number as their label, with the numeric label appearing above the band marker. Click on the "Label's" button. Click on the band to be labeled. Enter the text you wish to use as a label for that band or click on the "Mol. Weight or Amount" radio button to tell Gel-Pro to use these values as the label. Select the placement of the label, then click "OK." Gel-Pro displays the new band label in the overlay.

6.4.5 The Bands "Options" button provides several options for controlling the automatic band detection function. These options help limit the bands that are found, and control the way band peaks are found. Click the "Options" button. The Bands Options dialog box appears. Here, band height can be determined, what is displayed next to each band and what the band number represents can be changed. Other options include: Retain the largest # of peaks, Minimum Band Separation, and Center Peaks. For more details, refer to the Gel-Pro 6.0 Analyzer Start-Up Guide.

6.5 Lane Profiles

6.5.1 The Lane Profile dialog box will appear on the bottom right of the screen when "Lanes" option is selected from the ID-Gels tool palette. This dialog box allows the operator to plot the lane information on a line graph. The operator has the option to plot as many lanes as they need. The Lane Profile box corresponds to the selected lane on the scanned gel.

6.5.2 The bands in each lane correspond to the numbered peaks on the graph. Each peak represents a molecular weight value for that particular band (relative to the selected standard).

6.5.3 Using the "Zoom" buttons ([Z+] and [Z-]) enables a better view of the individual peaks. The operator can move the vertical brackets with the mouse to tighten the peak and increases the band resolution.

6.5.4 From the Lane Profile box, the operator can change the lane profile graph by adjusting the Baseline and changing the Band ID. For more information on this, refer to the Gel-Pro 6.0 Analyzer Start-Up Guide. Typically, one lane is plotted on the graph at a time and the Baseline is determined by selecting "From Image" and "Profile Minus Baseline."

NOTE: Before continuing to the next step, click on the lane that contains the molecular weight standard or IEF standard. This ensures that the standard is graphed in the lane profile dialog box, which is needed to generate the report at the end of this experiment.

6.6 Background Correction

6.6.1 The Background Correction option is used to define background lines parallel to the lanes, which can be subtracted from the lane profiles prior to calculating the amount of the bands. Background correction can correct uneven background intensities, and even compensate for irregularities due to uneven lighting, imperfect gels, non-uniform camera response, or minor optical imperfections.

Background correction does not modify the image. It defines a baseline for each lane profile. Gel-Pro then removes the background values from the optical density and intensity calculations. This results in more accurate molecular weight and amount calculations. Once the operator has corrected the background in the image, the Lane Profile, molecular weight, and amount calculations will be updated automatically to reflect the new values.

6.6.2 Click on the “Background” button from the ID-Gels tool palette and the Background Correction dialog box is displayed. Select “Background lines (From Image)." Select “Delete All," which removes the background correction lines from the image on the screen. Then select “Add Line," which will allow the operator to add vertical lines between the lanes on the gel image. Lines can be added wherever the operator specifies by moving the mouse cursor to the lane and clicking the left mouse button. Background lines should be positioned in areas of the image where there is little or no analytic material.

6.6.3 The “Always Show Background Lines” checkbox allows the operator to specify whether background lines should be shown or hidden in the overlay. If checked, background lines will be shown even after the Background Correction dialog box is closed. Typically, this box is left unchecked. Click “OK” when complete.

6.7 Molecular Weight Standards

6.7.1 The Molecular Weight Standards feature allows the operator to select the appropriate standard for calculating absolute molecular weights for the particular experiment. Depending on the type of experiment, Gel-Pro 6.0 allows the operator to select different units of measure to create the standard: base pairs or bp (DNA), bases (RNA), kilodaltons or kd (proteins), and isoelectric points or pI (proteins). Clicking on the “M.W. Standard” option from the ID-Gels tool palette shows the Molecular Weight Standard dialog box.

6.7.2 The operator can check the box at the bottom, which displays the molecular weight standards on the image in the workspace, even when the molecular weight dialog is closed if so desired.

6.7.3 Click on the "Select/Unselect" button and select the lane from the gel’s image containing the molecular weight standard.

6.7.4 Select the appropriate molecular weight standard from the drop-down list of available standards. For Tris-Glycine 4-20% SDS-Page Gels, the standard is “Mark 12 MW STD Tris-Glycine 4-20%," which includes 10 molecular weight markers. For NuPAGE 4-12% Bis-Tris Gels, the standard is “Mark 12 MW STD 4-12% Bis-Tris MES." This is Mark12 STD with 10 molecular weight markers plus an additional 2 markers at the end. For Western blots, “See Blue Plus2 STD 4-20% Tris-Glycine” is selected for Tris-Glycine gels, “See Blue Plus 2 STD NuPAGE 4-12% Bis-Tris MES” is selected for NuPAGE gels with MES running buffer and “See Blue Plus 2 STD for NuPAGE 4-12% Bis-Tris MOPS” is selected for NuPAGE gels with MOPS running buffer. For IEF Gels, the standard is “IEF Standard: Serva IEF Marker pH 3-10 gel."

6.7.5 By selecting “New," and typing in the name of the Standard, the operator can add and save additional standards in the standard pull-down box. The operator types in the molecular weight amount and clicks the “Add” button followed by “OK."

6.7.6 Other options available to the operator are as follows: Locate standard bands by manually positioning them, Auto Locate standard bands, Matching band for band, or allowing for one or two missing bands. Refer to the Gel-Pro 6.0 Start-Up Guide for more detailed information.

NOTE: The operator typically selects “Auto Locate’ the bands and “Match band for band."

6.7.7 Click “OK” when M.W. Standard is complete.

6.8 Slant Correction

6.8.1 The “Slant Correction” feature compensates for any accidental slanting or bending of the lanes in the gel image. This can be caused by high voltage used during electrophoresis, creating a “smile” effect where bands of identical molecular weight may appear at different distances from the wells (the origin) in the gel image. Slant Correction uses bands of similar molecular weight, found in at least two lanes, and links them with horizontal lines across the gel image. This feature is useful when bands of equal molecular weight migrate more in certain lanes than in others. Bands with identical molecular weights are now displayed as nearly congruent peaks.

6.8.2 Click on the “Slant” button in the ID-Gels tool palette to open the Slant Correction dialog box. To use the automatic slant correction facility, the operator must have identified at least two lanes as containing the molecular weight standards to be used for determining slant. Click on the “Auto Slant Lines” button. Slant lines are added automatically. They appear as horizontal lines drawn through the bands of equal molecular weight. Other Options include: “Add Slant Line” which allows the operator to add the molecular weight slant lines manually; “Delete Slant Line” which allows the operator to remove individual lanes; and “Always Show Slant Lines” which is a checkbox that allows the operator to specify whether slant lines should be shown or hidden in the overlay after the Slant Correction dialog box is closed. Click “OK” when finished with the Slant dialog box.

6.9 Results

6.9.1 Click on the “Results” button to view the results. The Amounts/Mol. Weights dialog box will appear. Data calculations will appear in the dialog box for each lane that was analyzed. Calculations include molecular weight and one of the following: Amount of the bands expressed in percent, relative abundance, or amount units; Absolute Integrated Optical Density (IOD), i.e., the volume of the band in the lane profile; Maximum IOD, i.e., the height of the band in the lane profile. Select “Show” from the top menu and check “Both."

6.9.2 “Loads” defines the mass loaded in each well. The operator can choose to load equal amounts of test substance or quantify each lane individually. Select “Loads” from the top menu and the Lanes Loading dialog box will appear. Highlight the selected lane and use the spin button to enter the appropriate amount or type in “100 ng” for each lane. Click “OK” when finished.

6.9.3 “Rel/bands” allows the operator to compensate for “lost” mass if you are determining band masses relative to the total mass loaded in each lane. This option calculates the relative abundance of band masses in each lane in relation to the total mass loaded in the lane, rather than the total mass detected. When this checkbox is selected, amount valves for each band have been increased proportionally such that the sum of values in each lane equals the total amount loaded in that lane. The operator can check the “Rel/bands” checkbox and select “Amounts" or the operator has the option of not checking the "Rel/bands," checkbox and just selecting "Amounts." If the second option is used, then the calculations will be made from the total mass detected in each lane.

7.0 Saving the Experiment

7.1 Click “File” then “Save” to save the updated Gel-Pro Image, using the QC Number for the title with a suffix if needed (example: QC-000000 NR or QC-000000 gel 1). Save images in the QC Public/Gel Pro 6/Images folder. Next, select “Save” from the ID-Gels tool palette. The Save Experiment dialog box is displayed. Type in the QC Number (with a suffix if needed) for the “Title," and the operator’s name for the “Experimenter." Then click on the “Save New” button (once the experiment has been saved, use the “Save” button for future saves). Save the densitometry data in the QC Public/Gel Pro 6/Densitometry folder."

7.2 Results can be exported to programs such as Microsoft Excel using the “DDE to Excel” function. Click on the “File” menu of the Amounts/Mol.Weights dialog box. Select “DDE to Excel”. The data from the results data table is copied into an untitled Excel spreadsheet. This can be helpful for compiling data into a report or for a presentation.

8.0 Generating and Printing a Report

8.1 Select “Reports” from the ID-Gels tool palette. A Report Generator box will appear. Select “File” from the tool bar and click on “Open." The Open Box will appear. Next to the “Look In” box, use the drop-down key and select “Reports” which is located in QC Public/Gel Pro 6/Reports folder. Double click on the “Reports” folder. A list of reports will appear. The first three are templates that are used to generate the report depending on the type of gel that was analyzed. They are as follows:

8.1.1 4-12% B-T NuPAGE Report, 4-20% T-G Report, and Novex IEF Report. Double click on the appropriate report.

8.2 The report template will appear, and it will need to be manipulated with the new results from the Gel-Pro 6.0 Analyzer. Begin by enlarging the screen. Select “Layout” from the tool bar. Click on “Update Data." The new data, including the gel image, the graph with the standard profile, and the results from the amounts calculated, will appear. Click on the gel image and resize it to fit on the right side of the report. Next, click on the graph box with the standard profile in it. Resize this box as well to fit in the middle-left portion of the report. Next, double click on the Label box. This will allow the operator the ability to enter new data pertaining to the gel. Enter “ID#” which is the QC number, “Product Name” which is the name of the sample analyzed, the “Lot #” of the sample, reference standard if there is one, and the “Gel #." Once this is complete, click on the Table box and resize to fit on the page.

8.3 To complete the Report, select “File” from the tool bar. Click on “Save As” and type the QC# in the File name box. Click on “Save” when finished.

8.4 To print the report, select “File” from the tool bar. Click on “Print” and the Print Box will appear. Determine which printer the report will be sent to and the number of copies and the click on “OK."

8.5 Close the Report Generator and Gel-Pro 6.0 Analyzer software.

9.0 Calculations

NOTE: Calculations performed will depend upon the Test Specification, Certificate of Analysis or Stability Protocol.

9.1 Percent Purity. After printing the Gel-Pro Analyzer Report, take the band amount found in the lane the sample was loaded in and divide it by the sum of that lane. This will give the percent purity for the sample requested. If the sum is out of 100, and “Rel/Bands” with an “amount” or “%” was selected in the Results section, then the calculation is already reported as a percent.

9.2 For a reduced antibody sample, there will typically be two dominant bands consisting of a heavy chain and a light chain. For both the heavy and the light chains, divide the band amount by the sum in the lane the sample was loaded. This will give the percent purity for each band. If the Results were reported as “Rel/Bands” with an “amount” or “%” (out of 100), then the Results calculated will already be reported in percent.

9.3 For an IEF gel, the sample submitter is requesting the sample pI (isoelectric point), the pH at which the protein has no net charge. After printing the Gel-Pro 6.0 Analyzer Report, the pI is the "molecular weight" for each band analyzed. If there is more than one band, the sample's pI is to be recorded as Band 1, Band 2, etc.

10.0 Documentation

10.1 Record the molecular weight for Non-Reducing and Reducing gels or pI value for each band on the BQC Test Request Form in the results section. See note in section 9.0.

10.2 Attach the Gel-Pro 6.0 Analyzer Report with the BQC Test Request, Form 22002-01 and the Gel Lane Assignment Form (with the gel taped to it). Stamp each page attached to the BQC Request Form, with QC Test Request #, Page __ of__ and Initials/Date Stamp. Fill out this information. Submit for BQC and BQA review.

11.0 References and Related Documents

11.1 Gel-Pro 6.0 Analyzer Start-Up Guide for Windows.

11.2 Gel-Pro 6.0 Report Generator User Guide for Windows.

11.3 Gel-Pro 6.0 Reference Guide for Windows.

12.0 Attachments

12.1 **Attachment 1** Example of Gel-Pro Analyzer v. 6.0 Report

13.0 Change Summary

[redacted]

Attachment 1

Example of Gel-Pro Analyzer v. 6.0 Report

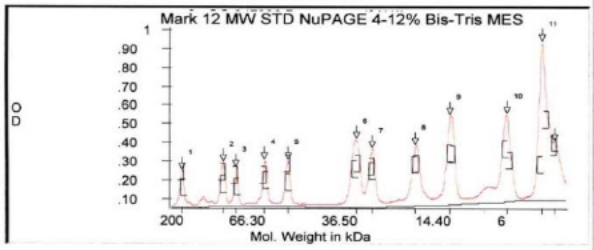
Gel-Pro Analyzer 6.0 Report ***SOP# 22906***

ID#: QC-000000

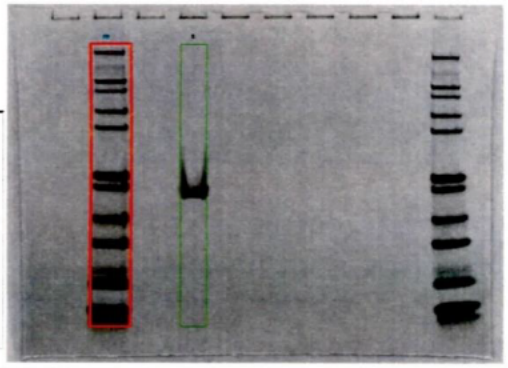
Product: Benzonase 100,000 Units
Lot# R00000000

Gel#: (NR)

M.W. Standard:



4-12% Bis-Tris NuPAGE Gel:



Lanes: / Bands	2 (mol.w.)	(amount)	4 (mol.w.)	(amount)
			Benzonase 100,000 Units BDP# R00000000	
1	200	2.0239	29.065	100
2	116.30	3.130		
3	97.40	2.1399		
4	66.30	3.4077		
5	55.40	3.2848		
6	36.50	11.489		
7	31	6.2657		
8	21.50	7.6168		
9	14.40	13.475		
10	6	13.914		
11	3.50	27.065		
12	2.50	6.1885		
Sum		100		100
In Lane		100		100

Performed By:_____**Date:**_____ **Checked By:**_____**Date:**_____