Gel Analysis Method for Determining the Distribution of Molecular Weight of Hyaluronan (HA) by Mini Agarose Gel Electrophoresis*

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Standard Operating Procedure

1. Purpose

1.1. The purpose of this SOP is to describe a reliable, reproducible method for determining the molecular weight distribution of the hyaluronan component of various materials using a mini agarose gel electrophoresis method.

2. Scope

2.1. Any personnel testing samples using the "Mini Agarose Gel Electrophoresis of Hyaluronan" Method at the Matrix Biology Institute.

3. Responsibilities

3.1. Any personnel performing the "Mini Agarose Gel Electrophoresis of Hyaluronan (HA)" method must be trained according to this procedure.

4. Materials

4.1. ImageMaster™1D Elite software (Amersham Biosciences version 4.10 or equivalent), Instruction manual for this software.

4.2. A densitometric scan of the mini agarose gel produced in the Procedure "Mini Agarose Gel Electrophoresis of Hyaluronan (HA)", section 6.9.

4.3. Working copy of an Excel Spreadsheet "Gel Analysis Spreadsheet".

5. Definitions (not applicable)

6. Procedures

6.1. Gel Analysis

6.1.1. Open the Imagemaster program and click "new" to start a new experiment then pull up the scan from the appropriate location. Click on the "Fit image to window" button. To adjust the brightness and contrast of the image click on the "contrast and color " icon on the top tool bar.

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6.1.2. Create lanes

6.1.2.1. Click on “create lanes” icon. Use the following settings: manual, create lanes, 10 lanes, 90% width.

6.1.2.2. The first vertical icon in the window will be highlighted. Put the cursor in the upper left corner of the gel and draw one box around all the lanes. The lanes will isolate and expand as they are drawn out with the curser.

6.1.2.3. Click the third vertical icon in window. Edit any individual lanes by clicking on the lane or using the arrow buttons, then if necessary, place markers to bend lane to the appropriate shape that conforms to the actual shape of the lane.

6.1.2.4. Save all results using the number of the gel as the name of the file, then the next consecutive number, for example, minigel 001-10.

6.1.2.5. Label the lanes by right clicking on lane number and type in heading desired.

6.1.2.6. Save all results.

6.1.2.7. For other appropriate modifications see the instruction manual or help file.

6.1.3. Subtract the background

6.1.3.1. Click “background” icon. Select ” Image Stripe” method.

6.1.3.2. Click on first lane containing a standard or sample. Put cursor in an adjacent empty lane, hold down left mouse button and draw an image stripe of blank lane. Watch graph of the lane image and adjust image rectangle until the background is erased and the right side of the image line sits on the origin.

6.1.3.3. Repeat 6.1.3.2 for each lane containing a standard or sample.

6.1.4. Detect the bands

6.1.4.1. Click on the “band detection” icon. Move green box to band desired to detect peak.

6.1.4.2. Manually select peak apex using cursor and right click to confirm. Move to next band using arrow buttons on left of window and choose peak apex and confirm. Continue until all peaks have been identified. When the peak has been established, the ”data” box will show the position of that peak. Record the position of each peak including the standards in an appropriate place.

6.1.5. Save the results

6.1.5.1. Save all results using the number of the gel as the name of the file, then the next consecutive number, for example, minigel 001-10.

6.1.6. Reports (optional)

6.1.6.1. Click on the “report” icon.

6.1.6.2. Click on the “batch printing” box.

6.1.6.3. Check the “print gel report (current gel only)” box

6.1.6.4. Select all lanes.

6.1.6.5. Select under QC Report: “none”.

6.1.6.6. Select under MW Calibration Report: “none”.

6.1.6.7. Click the “Print Reports Now” box.

6.1.6.8. Click the “OK” box.

6.1.7. Excel analysis

6.1.7.1. Open a working copy of the “Gel Analysis spreadsheet” used to analyze the gel data. Erase all data in columns D through I of the “DATA” page if present.

6.1.7.2. Save file in appropriate folder using the name of the gel (the gel number), for example, minigel 001.

6.1.7.3. Replace the position values for the standards in column F on the “STANDARDS” page with the values for the current gel being analyzed. They can be found on the Imagemaster report pages printed in section 6.1.6 or what you have recorded when detecting each band in 6.1.4.2.

6.1.7.3.1. For sample MW range of about >200,000, use appropriate Hyalose, LLC standard ladders or individual standards above 200,000.

6.1.7.3.2. For sample MW range of about <200,000, use appropriate Hyalose, LLC standard LoLadder, only the lowest 3 values and/ or individual standards of MW < 200,000. At least three standards are required for the slope.

6.1.7.4. The slope can be viewed in the graph of the standard positions along with the standard deviation on the “STANDARDS” page. The standard deviation of the line for the standards should be 0.95 or greater. If not, a single standard value may be removed from the calculation, and the line and standard deviation recalculated using at least three points.

6.1.7.5. Enter the new values for m and b from the equation in the graph of standards in the appropriate box in column H on the “STANDARDS” page. Record the standard slope in an appropriate place or print out the page.

6.1.7.6. Resave data as per step 6.1.7.2.

6.1.7.7. Place the sample names for the current samples being analyzed in the appropriate columns D through K of the “DATA” page.

6.1.7.8. Copy the lane profile from Imagemaster for each sample into the appropriate columns D through K, starting in row 3, of the “DATA” page of the Excel program using the same order as the sample names placed in appropriate columns D through K from step 6.1.7.7.

6.1.7.9. Eight lanes may be entered in a single file.

6.1.7.10. Save all results as per step 6.1.7.2.

6.1.7.11. The graph of the distributions of relative absorbance vs MW is on the far right of the “DATA” spreadsheet in both logarithmic and linear scales (located under the logarithmic graph). They are displayed using 80% smoothed data, (columns U through AB). Normalized data is also calculated in columns AD-AK and they can be exchanged in the source data of the graphs if normalized data is desired. Change any existing parameters in the graphs to fit the data such as scale for both x and y axis. These graphs can be transferred to a powerpoint program or other appropriate program for preparing a results page if desired.