Image Lab™ Software
How to Obtain Stain-Free Gel and Blot Images

Instructions

BIO-RAD

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If the Gel Doc™ XR+ or ChemiDoc™ XRS+ systems are used to conduct quantitation with Bio-Rad's stain-free gel technology, Bio-Rad recommends performing the flat field calibration with the orange fluorescence reference plate (cat. #170-8008) after installing Image Lab™ software, version 5.1 or higher.

To perform the calibration using the orange fluorescence reference plate, choose **Edit** > **Instrument Setup** in the Image Lab software and select **Reset** next to the flat field in the dialog box. Image Lab will guide you through the 5 min process.

Obtaining Stain-Free Gel Images

In order to visualize protein bands using stain-free technology on gels and blots (if applicable), stain-free gels must be activated after electrophoretic separation is complete. Once activated, the same protein bands can be visualized (without any further activation) on blotting membranes after transfer. Thus, stain-free technology enables one to monitor electrophoretic separation and transfer effeciency.

Instructions

1. Immediately after electrophoretic separation is complete, remove the gel from the cassette and place it directly on a stain-free enabled Bio-Rad UV transilluminator (ChemiDoc™ MP, ChemiDoc XRS+, Gel Doc XR+) or stain-free tray (Gel Doc™ EZ); no fixation or rinsing steps are required.

   Note: Do not allow gel to soak in water or other solution after separation before activation and imaging. Soaking an unactivated gel allows the stain-free moiety to diffuse out of the gel. Once activated, the gel can sit in buffer or water as usual.

   Note: Do not place any material, such as plastic film, between the gel and the plate or tray.

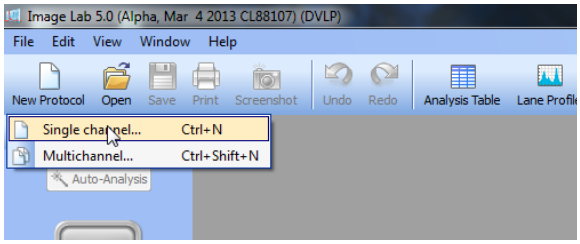
2. Open Image Lab software.

   a. Version 4.1 or later for Gel Doc EZ or ChemiDoc MP.

   b. Version 5.1 or later for Gel Doc XR+ or ChemiDoc XRS+.
   Download Image Lab version 5.1 at www.bio-rad.com/info/imagelabsoftware.

3. To acquire a stain-free gel image (Gel Doc EZ, Gel Doc XR+, ChemiDoc XRS+), configure the acquisition settings by selecting **New Protocol**.

   a. Select **New Protocol** > **Single Channel** for ChemiDoc MP.

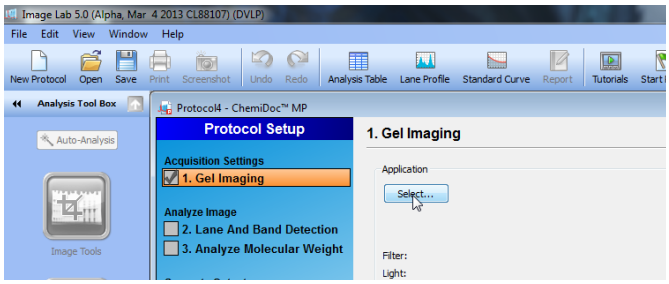


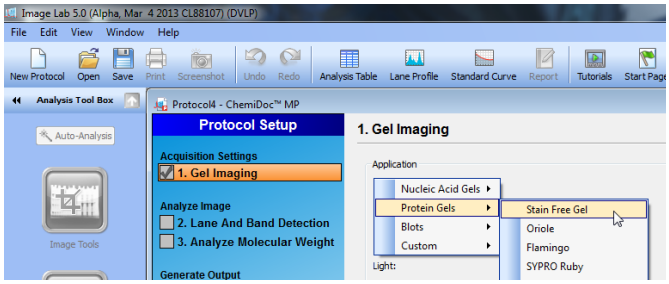
4. In the Application pane, click **Select** and then **Protein Gels** > **Stain Free Gel**. Now select the gel activation, depending on the application.

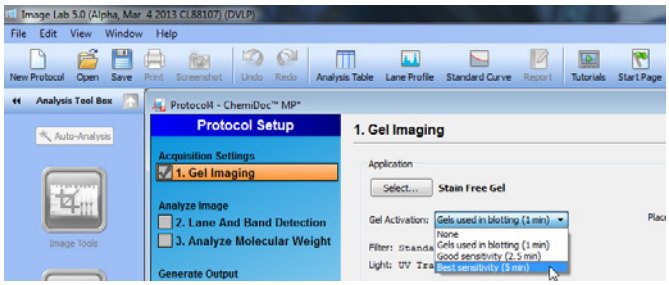
   a. 1 min activation provides sufficient UV activation for gels used in western blots.

   b. 2.5 min activation provides a good balance between time required for UV activation and signal intensity.

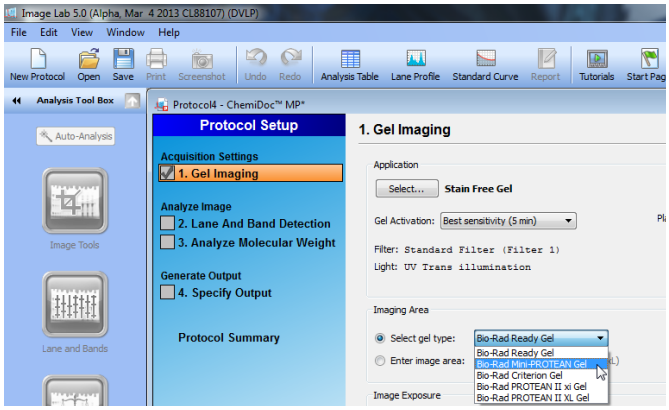
   c. 5 min activation provides maximum signal intensity.



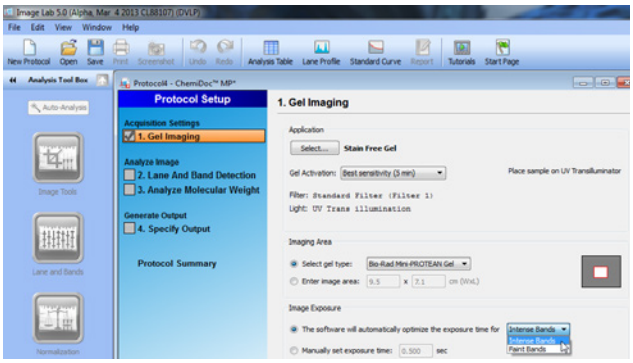




5. In the Imaging Area pane, select from the list of Bio-Rad gels or enter the image area dimensions manually. This option is not necessary when using the Gel Doc EZ, as the entire area is imaged automatically.

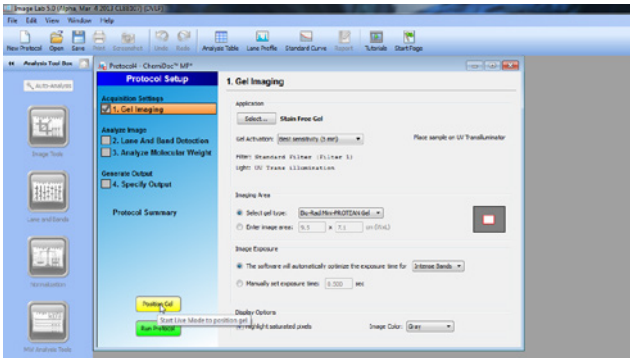


6. In the Image Exposure pane, select from one of the following options:
   a. **Auto Exposure** — this setting estimates an optimal exposure time and ensures the best use of the dynamic range.
      i. **Intense Bands** — optimizes exposure for all bands.
      ii. **Faint Bands** — a longer exposure time is used to make faint bands more visible, but more prominent bands might be overexposed.
   b. **Manual Exposure** — use this setting to manually override automated imaging. This setting is often used to duplicate an exposure time in order to compare band intensities from different gels.

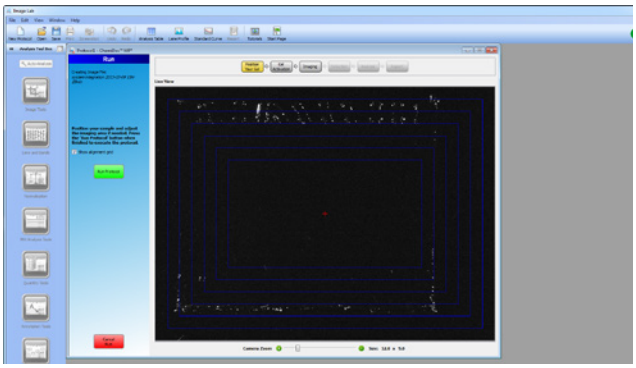


7. In the Display Options pane, keep the default settings. If using the Gel Doc EZ, proceed to step 10.

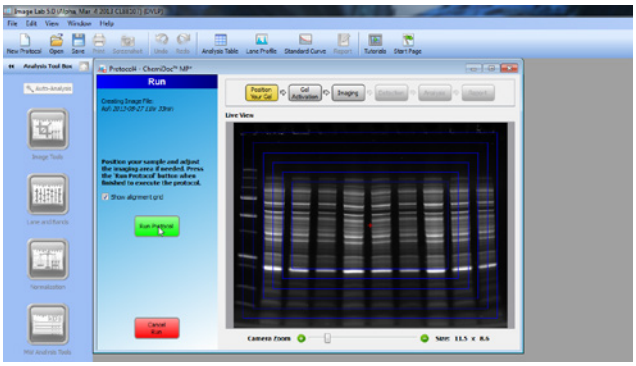
8. Select **Position Gel**. The acquisition pane will now display a live view of the gel.



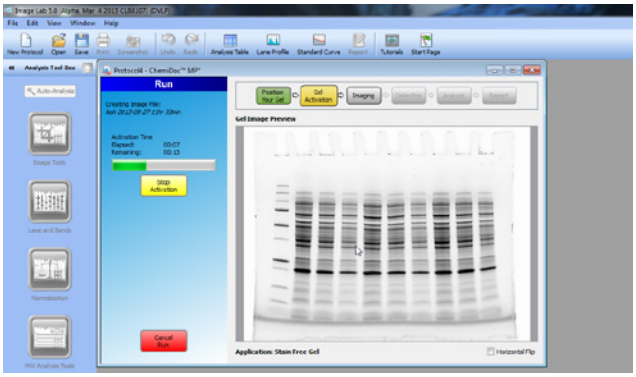
9. Make sure the gel is correctly positioned in the viewing area. Adjust the zoom if necessary and/or manually align the gel.



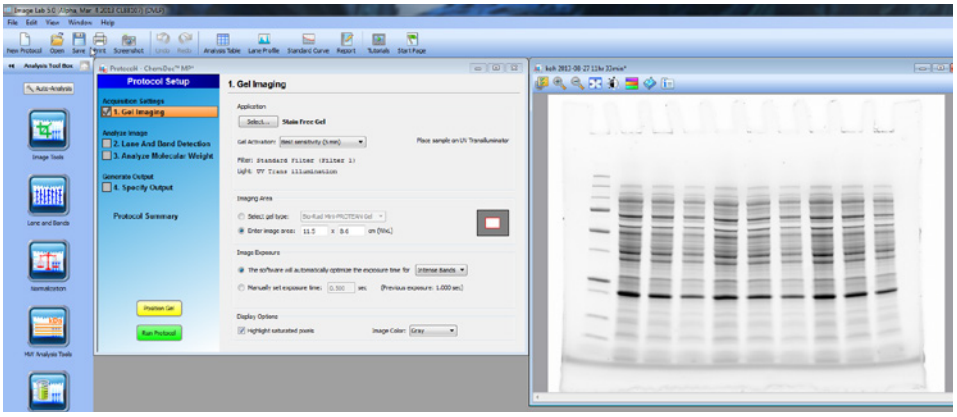
10. To start the stain-free activation and image acquisition, select **Run Protocol**.



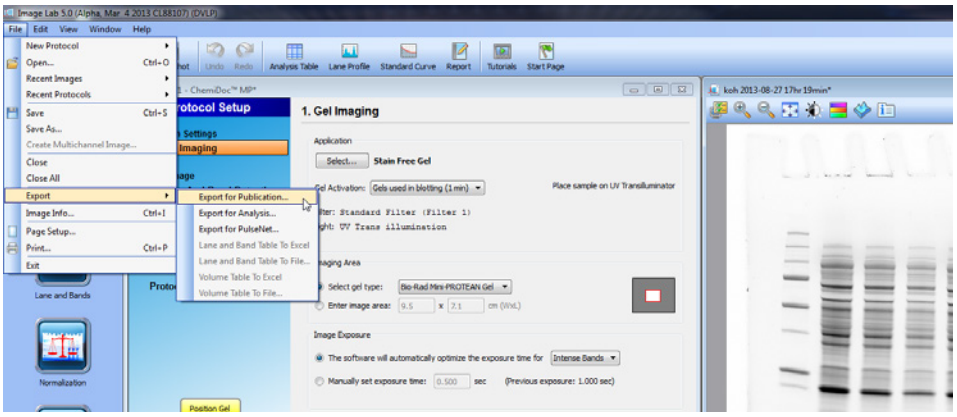
11. The Gel Image Preview pane will now display the stain-free gel undergoing activation in real time.

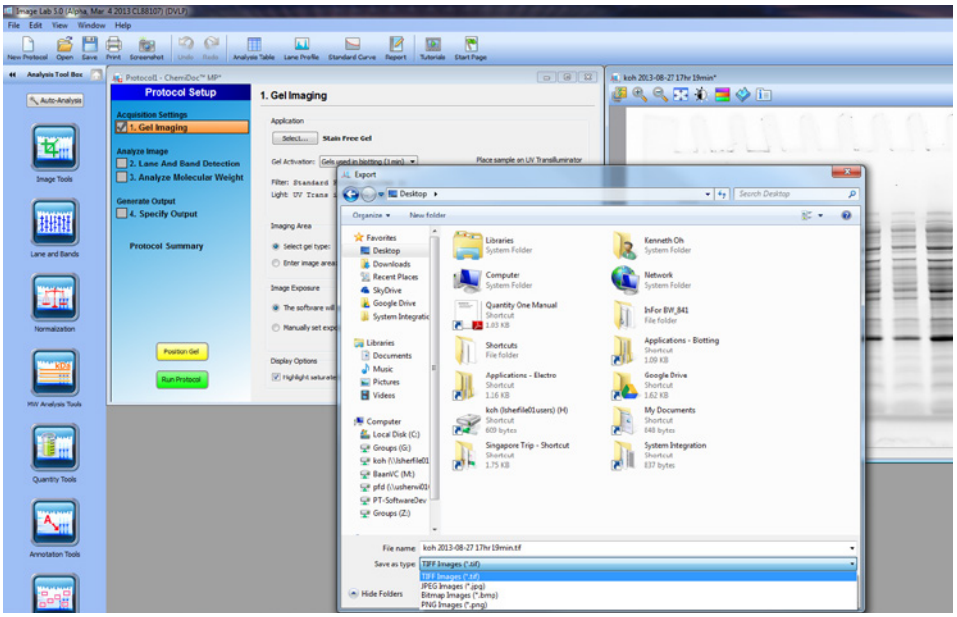


12. Once activation is complete, an image of the stain-free gel is captured, based on the image exposure time selected.



13. The gel image can now be exported in a variety of image formats (TIFF, JPEG, bitmap, or PNG).





Obtaining Stain-Free Blot Images

Instructions

1. Once the blotting step is complete, remove the blot and place it directly on a stain-free enabled Bio-Rad UV transilluminator plate (ChemiDoc MP, ChemiDoc XRS+, Gel Doc XR+) or stain-free tray (Gel Doc EZ).

   Note: No fixation or rinsing steps are required. Membranes are more transparent to UV illumination when wet. For optimal imaging, keep membranes wet with transfer buffer or water. Transfer buffer or water also helps minimize air bubbles that can be trapped under the membrane.

   Note: Do not place any material, such as plastic film, between the blot and the plate or tray.

2. Open Image Lab software.

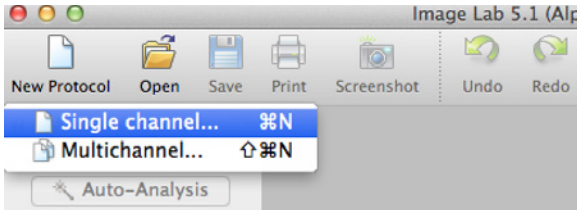
   a. Version 4.1 or later for Gel Doc EZ or ChemiDoc MP.

   b. Version 5.1 or later for Gel Doc XR+ or ChemiDoc XRS+.
   Download Image Lab version 5.1 at www.bio-rad.com/imagelabsoftware.

3. To acquire a stain-free blot image, configure the acquisition setting.

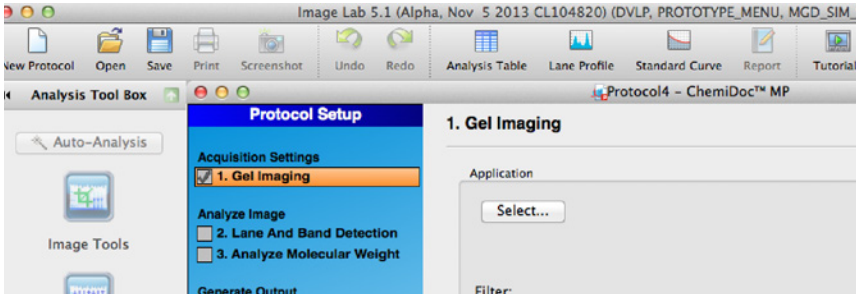
   a. Select **New Protocol** for Gel Doc EZ, Gel Doc XR+, and ChemiDoc XRS+.

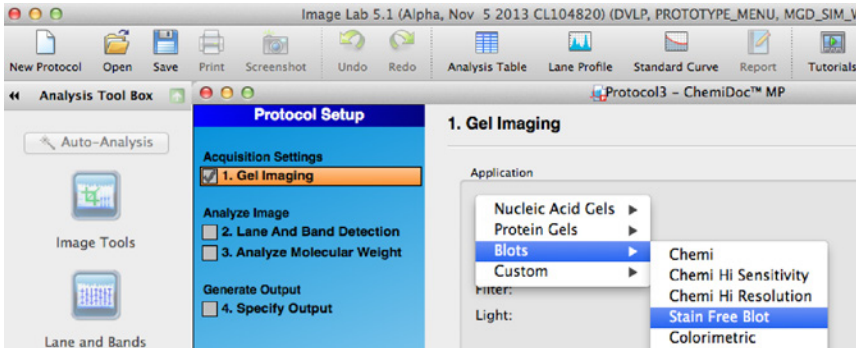
   b. Select **Single channel** for ChemiDoc MP.



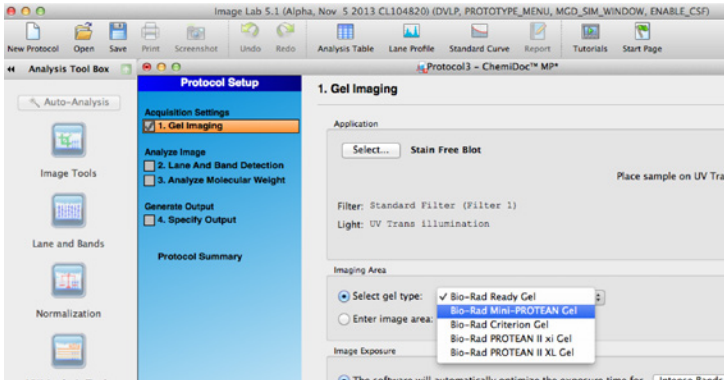
4. In the Application pane, click **Select** and then **Blots** > **Stain Free Blot**.

   Note: For the Gel Doc EZ, Stain Free Blot is under the Protein Gels Application

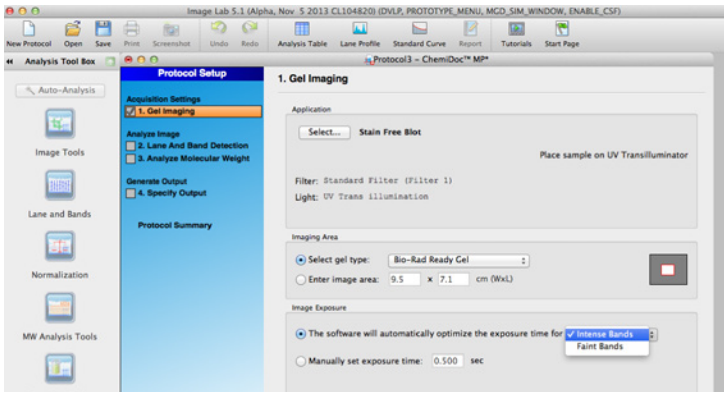




5. In the Imaging Area pane, select from the list of Bio-Rad gels or enter the image area dimensions manually. This option is not necessary when using the Gel Doc EZ, as the entire area is imaged automatically.

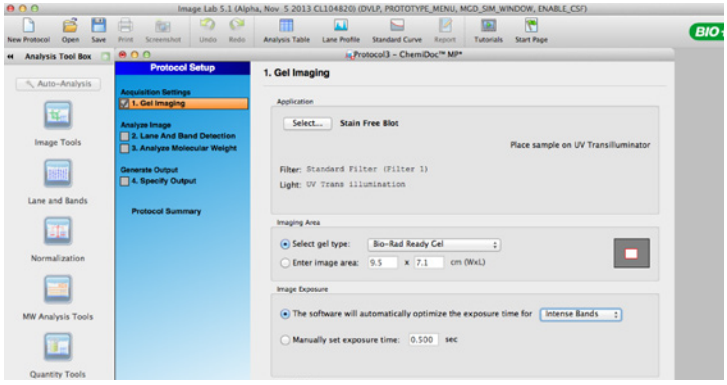


6. In the Image Exposure pane, select from one of the following options:
   a. **Auto Exposure** — this setting estimates an optimal exposure time and ensures the best use of the dynamic range.
      i. **Intense Bands** — optimizes exposure for all bands.
      ii. **Faint Bands** — a longer exposure time is used to make faint bands more visible, but more prominent bands might be overexposed.
   b. **Manual Exposure** — use this setting to manually override automated imaging. This setting is often used to duplicate an exposure time in order to compare band intensities from different gels.

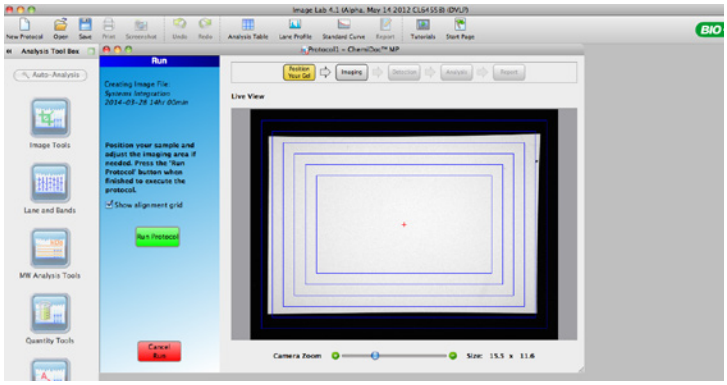


7. In the Display Options pane, keep the default settings. If using the Gel Doc EZ, proceed to step 10.

8. Select **Position Gel**. The acquisition pane will now display a live view of the blot.



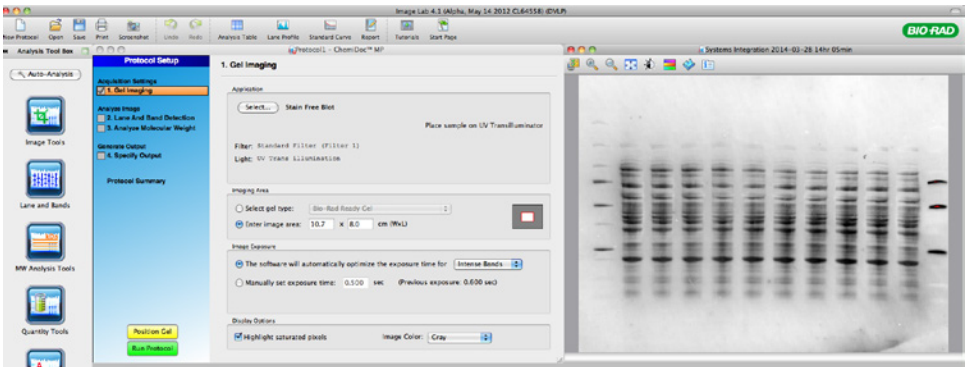
9. Make sure the blot is correctly positioned in the viewing area. Adjust the zoom if necessary and/or manually align the blot.



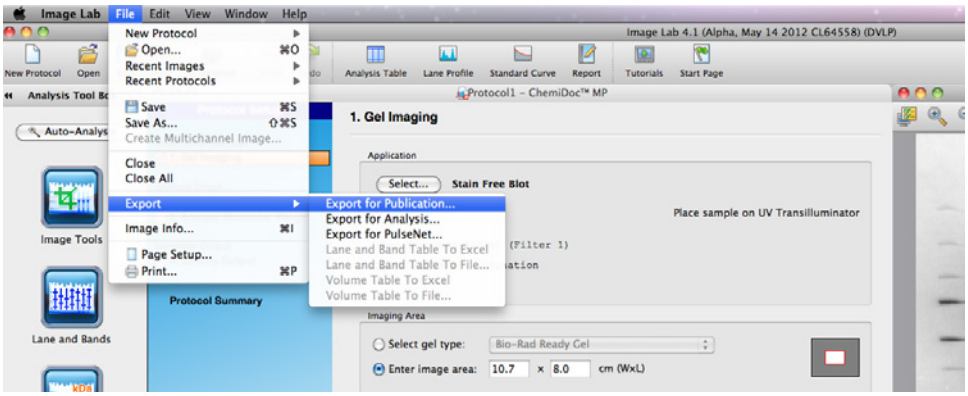
10. To start the stain-free blot and image acquisition, select **Run Protocol**.

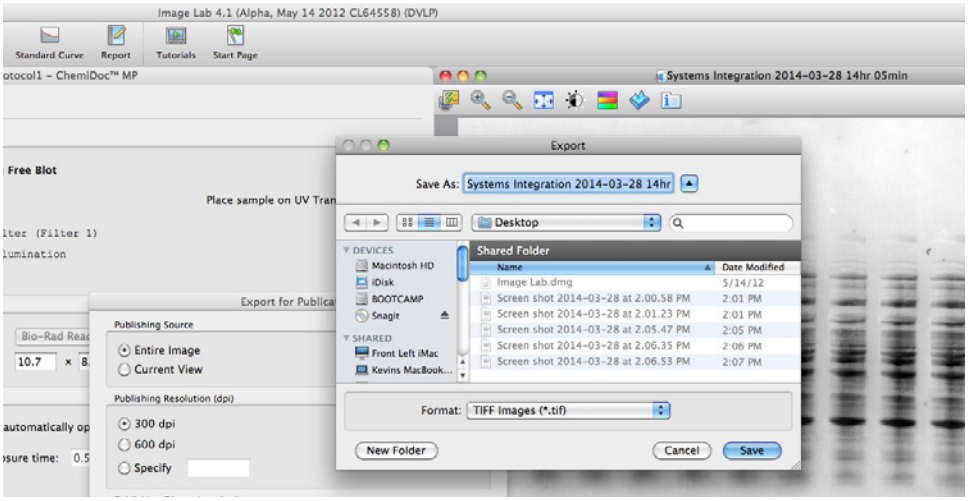
11. The Blot Image Preview pane will now display the stain-free blot undergoing image acquisition in real time.

12. Once image acquisition is complete, an image of the stain-free blot is captured, based on the image exposure time selected.



13. The stain-free blot image can now be exported in a variety of image formats (TIFF, JPEG, bitmap, or PNG).





Notes and Tips

If desired, stain the gel with any TGX-compatible stains after stain-free imaging. Certain stains eliminate detection capability if used prior to imaging.

Wet the UV transilluminator with 2–3 ml of water prior to gel placement in order to avoid tearing.

If uneven stain-free development is observed, briefly rinse the gel with water after electrophoresis. However, prolonged rinsing may affect the intensity of the stain-free signal.

Since gels are transparent, what shows in the Live View window of ImageLab for ChemiDoc MP, XR+, and XRS+ instruments are white dots that define the edges of the gel.

For more detailed instructions, refer to the Gel Doc EZ Stain-Free Sample Tray Instruction Manual (#10019634), the ChemiDoc MP System with Image Lab Software Instruction Manual (#10022469) and the Mini-PROTEAN® Precast Gels Instruction Manual and Application Guide (#1658100). These bulletins, as well as a library of other manuals, guides, and technical information are available at **www.bio-rad.com**.

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