V3 Western Workflow™ Protocol

Separate Proteins

Rapidly separate proteins with TGX Stain-Free™ precast gels

Procedure

1. Load 5 µl of protein standard; see tips below for specific recommendations.
2. Load samples.
3. Run gels at 300 V for 15–20 min for Mini-PROTEAN® gels and 18–25 min for Criterion™ gels.

Tips

- For all gels, load 5 µl of a tenfold dilution of Precision Plus Protein™ WesternC™ standards (10 µl of standards diluted in 90 µl of sample loading buffer) on the gel (visible in chemiluminescence and fluorescence applications)
- For a prestained standard, use Precision Plus Protein All Blue standards (visible when running the gel, but not when the gel is imaged in the stain-free channel; it will show as a negative stain)
- For molecular weight estimation, use Precision Plus Protein Unstained standards (visible in stain-free images, but not visible during electrophoresis)

Visualize Separation

Immediately visualize separation using stain-free technology and the ChemiDoc™ MP imager

Procedure

1. Remove the gel from the cassette and immediately activate the gel on the ChemiDoc MP imager.
2. Open Image Lab™ software 4.1 and select **New Protocol > Single Channel**.
3. In **Application**, select **Protein Gel > Stain Free Gel**.
4. Set **Gel Activation** to **gels used in blotting (1 min)**.
5. In **Imaging Area**, select **Gel Type**: either **Bio-Rad Mini-PROTEAN Gel** or **Bio-Rad Criterion Gel**.
6. In **Image Exposure**, select **The software will automatically optimize the exposure time for** and select **Intense Bands**.
7. Click **Position Gel** (yellow button) to ensure the gel is centered within the selected imaging area.
8. Click **Run Protocol** (green button) to begin activation and imaging.

Tips

- Note the image exposure time on the protocol screen to visualize the gel after transfer
- The **Intense Bands** exposure setting is a good starting point, but exposure time may need to be manually adjusted on some sample types to avoid signal saturation of the stain-free bands

Transfer Proteins

Use the Trans-Blot® Turbo™ system for rapid and efficient protein transfer

Procedure

1. Open a Trans-Blot Turbo transfer pack; place the pad with the membrane in the cassette base.
2. Place the gel on top of the membrane, place the top pad on the gel, and roll out bubbles.
3. Place the lid on the cassette base and lock the lid.
4. Insert the cassette into either instrument bay.
5. Start the transfer by selecting **TURBO**, choose the gel size specific to the experiment, and press **RUN**.

Tips

- Immun-Blot® low fluorescence (LF) PVDF membranes reduce background for fluorescent and stain-free applications; to use, dip in methanol for 30 sec, equilibrate in water for 1 min, then replace the membrane in the Trans-Blot Turbo transfer pack with the equilibrated LF PVDF membrane
- The membrane will be warm after transfer; quickly transfer the membrane to water or buffer to prevent the membrane from drying out

BIO-RAD

Verify Transfer

Verify high-quality transfer by instantly imaging proteins on the membrane

Procedure

1. Image the blot by selecting the **Stain Free Blot** application; position the membrane before running the protocol.

Fluorescent Immunodetection

- Immunodetect the membrane using a standard protocol; avoid using blue excitable fluorophores with stain-free detection
- Image by selecting **New Protocol > Multichannel**; set one channel to **Stain Free Blot** and the other channels to the fluorophores of interest; position the blot prior to image capture and save the image

Chemi Detection

- Immunodetect the membrane using a standard protocol, then image by selecting **Stain Free Blot**; save the image for normalization
- Add the substrate, then image the blot by selecting a **Chemi** protocol; position the blot prior to image capture and save the image

Tips

- Image the gel after transfer by selecting the **Stain Free Gel** application and set the activation time to **None**; manually set image exposure time to match the pre-transfer gel (step 2)
- Match the transform settings between the pre- and post-transfer gel images using **Window > Imitate Transform**

Validate Western Blot

Perform multiplex imaging and validate results by total protein normalization on the ChemiDoc MP imager

Procedure

1. Select **Normalization** from **Analysis Tool Box**.
2. Select **Yes** to detect lanes and bands (select defaults unless detection parameters are known).
3. Select **Stain Free Blot** as channel for normalization.
4. Make sure **Total lane protein** radio button is selected (default setting).
5. Select green up arrow on top, then select **Lanes and Bands**; Use **Lane Profile** on upper tool bar to adjust background subtraction for profile consistency.
6. Return to green up arrow and go to **MW Analysis Tools** to designate any MW standard lanes.
7. View normalized volumes in the **Analysis Table** on upper tool bar.

Tips

- For accurate quantitation, adjust the lanes to include the entire width of all bands
- Lanes should be the same size and span the same region without touching adjacent lanes