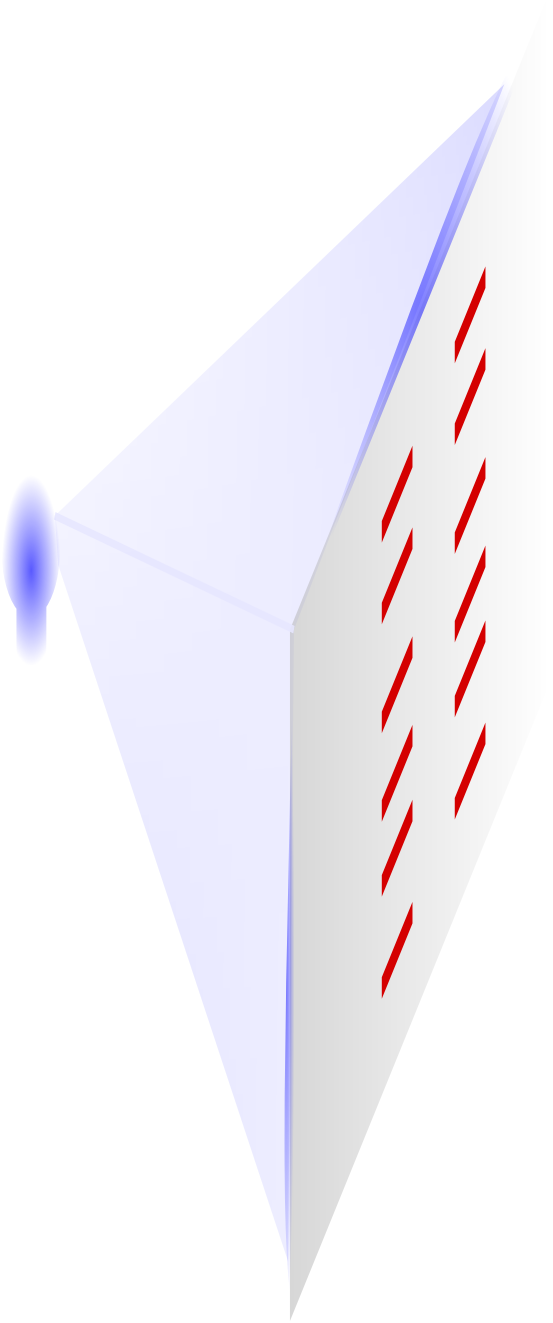


GlowPage System

Enzyvaa

User Manual

A. Product Description:

GlowPage System is a multi component system that allows you to perform SDS-PAGE in 20-25 min, protein visualization in 2 min, transferring protein onto transfer membrane in 5-7 min and finally total protein normalization. Its components include:-

1. **GlowPage (10%) Gel:** Allows you to cast acrylamide gel equivalent to 10% Laemmli Page based Gels but offers additional advantages such as minimizing protein modification-degradation problem and also protein resolution in 20-25 min. Other format of gel shown below can be selected based on your target protein size and optimum separation range of each gel type

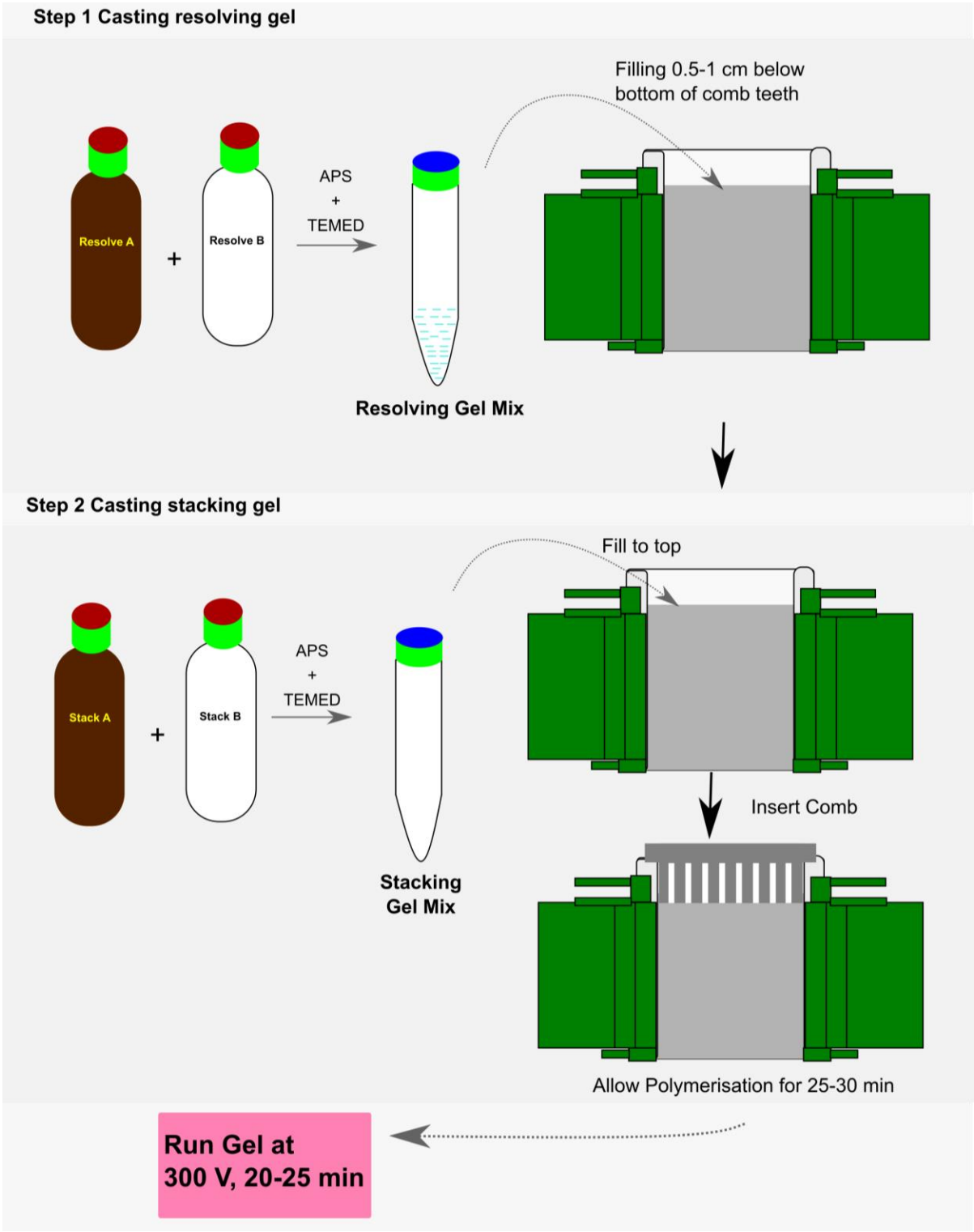
Gel Percentage /Gel Type	Optimum Separation Range	Product No.
7.5%	40-200 kDa	GPH-7.5P
10%	30-150 kDa	GPH-10P
12%	20-120 kDa	GPH-12P

Furthermore, these gels allow you to visualize protein bands by just UV exposure for 2 min due to the presence of proprietary protein dye incorporated in GlowPage Gels.

2. **4X GlowPage Loading Dye:** Loading dye specially formulated for preparing protein samples to run on GlowPage Gels

3. **GlowTransblot Buffer (5X):** Allows you to transfer protein in 5-7 min on commonly used Trans-Blot® SD Semi-Dry Transfer Cell (Biorad) with use of Thick blot paper

B. GlowPAGE Gel Workflow



Kit Components

1. GlowPage (10%) Gel
2. 4X GlowPage Loading Dye
3. Thick Blot Paper
4. GlowTransblot Buffer (5X)
5. PVDF membrane (0.45 µM)

C. Detailed protocol

1. Preparation of GlowPage Gel Mix

GlowPage (10%) Gel pack contains **Stack A, Stack B** for casting stacking gel and Resolve A and Resolve B for casting resolving gel. To prepare mix for casting these gels, follow the protocol as mentioned below:-

1.1 Prepare **Resolving Gel mix** by combining **Resolve A** and **Resolve B** in equal proportion (As per below Table). Similarly, prepare **Stacking gel mix** by combining **Stack A** and **Stack B** in equal proportion. Add freshly prepared APS and TEMED in those prepared mixes.

	0.75 mm Glass Plates (Biorad)		1 mm Glass Plates (Biorad)		1.5 mm Glass Plates (Biorad)	
	Stacking Gel Mix	Resolving Gel Mix	Stacking Gel Mix	Resolving Gel Mix	Stacking Gel Mix	Resolving Gel Mix
Resolve A	-	2 ml	-	2.5 ml	-	3.5 ml
Resolve B	-	2 ml	-	2.5 ml	-	3.5 ml
Stack A	1 ml		1 ml		1.5 ml	
Stack B	1 ml		1 ml		1.5 ml	
10% APS	20 µl	40 µl	20 µl	50 µl	30 µl	70 µl
TEMED	4 µl	4 µl	4 µl	5 µl	6 µl	7 µl

Caution: *If using high purity TEMED and APS, then above proportion of TEMED and APS will work as indicated. Otherwise, optimize TEMED and APS proportions around the recommended concentration.*

2. Casting of GlowPage Gels

2.1 Assemble glass plates on the Casting stand as per manufacturer instructions.

2.2 Add resolving gel mix from middle of assembled cassette to a height having a distance of 0.5 to 1 cm from edge of comb teeth

2.3 Add stacking gel mix slowly & continuously from middle of the assembled cassette

Note: *Do not overlay with* ***water or isopropanol or isoamyl alcohol*** *after adding resolving gel mix. Start adding stacking gel mix simultaneously after adding resolving gel mix. Be careful to add stacking gel mix from middle of gel slowly.*

2.4 Allow the gel to polymerise at room temperature for 30-45 min

Note: Low *temperature of surroundings in winter slightly* ***increases polymerization time****. Be careful and verify it with an aliquot of gel mix kept in parallel for visual verification of polymerization*

3. Sample & Reagent Preparation

3.1 Sample Preparation

Reagents	Reduced Sample	Non-Reduced Samples
Sample	**5 µl**	**5 µl**
4X GlowPage Loading Buffer	**2.5 µl**	
D.W	**2.25 µl**	**2.5 µl**
β-mercaptoethanol (14.3 M)	**0.25 µl**	**-**
4X Native Gel Loading Buffer		**2.5 µl**

Note: *Instead of β-mercaptoethanol, 1 µl of DTT (500 mM) can be used in the reduced sample preparation to achieve a final DTT conc. of 50 mM.* The same gel can be used for Native Page with following non reduced samples preparation guidelines as per above table.

3.2 Reagents Preparation

1X Running Buffer Preparation

Reagents	Amount
Tris-base	**3.02 g**
Glycine	**14.41 g**
SDS	**1 g**
Deionized water make up	**1000 ml**

Note:

- MQ (Trade Name: Merck) or any Type 1 water with > 18 milli Ohm (Resistance) are used for preparation of running buffer.
- Running buffer can also be made as 10X and diluted to 1X before use

4. Running GlowPage Gels

4.1 Assemble the cassettes into the SDS-PAGE running module of Supplier. Fill inner and outer chamber of the module with running buffer.

Note: *Fill outer chamber almost to the position where resolving gels starts. It helps in dissipation of heat generated during run and helps to some extent in bands sharpening.*

4.2 Load the prepared samples into the wells.

Note: *The maximum height of the sample in the well should be half of the height of the stacking gel (e.g if stacking gel height is 1 cm then maximum sample load height should be 0.5 cm. This ensures band sharpness, even for diluted protein samples*

4.3 Run the gel at 300V or 200 V or 100 V as per experimental need (For reference see below table)

Note*: We recommend 300 V for routine SDS-PAGE. For heat sensitive applications run gels at 100 V or 200 V. Importantly, 4 Gels can also be run simultaneously at 300 V.*

		100 V (Low Voltage)	**200 V (Standard)**	**300 V (Fast Mode)**
Run Time		90-100 min	30-40 min	20-25 min
Expected Current (Per Gel)	**Initial**	15-25 mA	25-50 mA	50-75 mA
	Final	5-10 mA	20-30 mA	40-70 mA
Expected Temperature		25 ºC	25-35 ºC	30-42 ºC
Lower Bufer Vol. (For 2 Gels)		550 ml	550 ml	800 ml
Lower Buffer Vol (For 4 Gel)		800 ml	800 ml	800

4.4 Terminate gel run after 20-25 min or when dye front reaches the bottom of gel.

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5.0 Protein Imaging with GlowPage Gels

GlowPage gels can be used for visualization of protein bands after completion of electrophoresis. Follow the protocol as discussed below:-

5.1 UV exposure of Gels

i) Take out the polyacrylamide gel from glass plates after completion of electrophoresis
***Caution**: After removing gel from the cassettes, do not submerge gel in any solution before next step. It causes decrease in effective concentration of protein dye and thus results in decreasing the sensitivity of detection.*

ii) Expose the polyacrylamide gel for **2-5 minute** under UV light to form Dye- protein conjugates. This step can be performed in the Gel Doc or UV illuminator (302 nm).
Note: ***2 min of UV exposure generally gives you a good amount of sensitivity which can be increased further by increasing the exposure time to 5 min. importantly, if the downstream application is western blotting, then 1 min of exposure is generally recommended.*** *If using Biorad Gel Documentation System for imaging, then read instruction in section 5.3 (***UV exposure and Imaging GlowPage simultaneously using ImageLab Software in Biorad Gel Documentation system**)

5.2 UV imaging

5.2.1 Imaging of UV exposed GlowPage Gel:

The photo-activated protein in polyacrylamide gel can directly be visualized upon UV exposure in gel documentation system commonly used for DNA imaging applications.

- Follow the same protocol as used for DNA agarose gel imaging

5.2.2 Imaging of Nitrocellulose or PVDF membrane:

For detection of protein on PVDF or nitrocellulose membrane after transfer from GlowPage Gels, no additional conjugation step is required. The membrane can directly be exposed to UV light in gel doc system and image can be acquired. **Care must be taken that the polyacrylamide gel photo-activation (step ii) must have been performed on the polyacrylamide gel before moving for protein transfer on membrane.** Otherwise protein will not be detectable on the membrane upon UV exposure. Also, it important to keep the membrane wet all the time, otherwise it will increase background level.

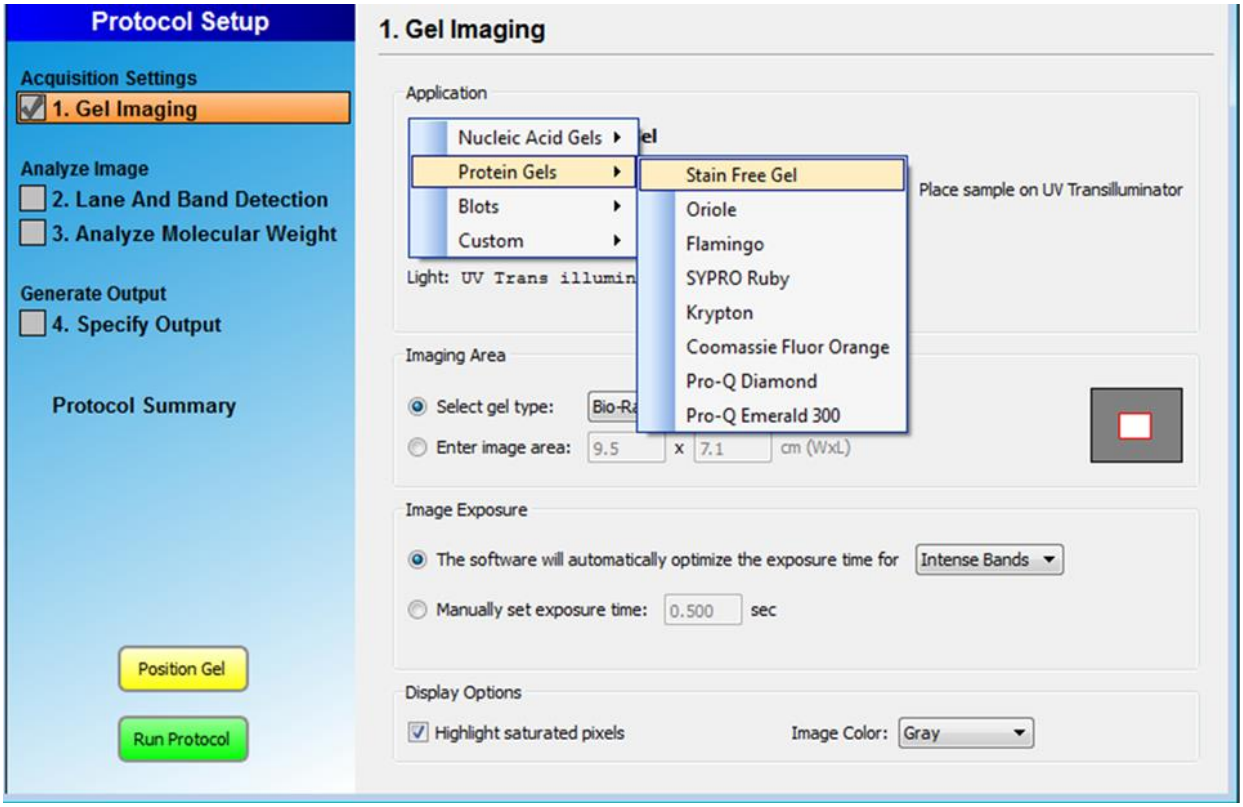
Note: PVDF membrane should be used if you want to image the transfer membrane during western blotting. More preferably, PVDF membranes with low fluorescent background give very good results. We do not recommend use of internally supported nitrocellulose membrane for imaging protein bands on transfer membrane due to their low transmittance property. Although, internally supported nitrocellulose membrane are compatible with GlowPage System and can be used if imaging transfer membrane is not needed.

5.3 UV exposure and Imaging GlowPage Gel using ImageLab Software in Biorad Gel Documentation system

Some Gel Doc manufacturer such as Bio-Rad has pre-set protocol with pre UV exposure before imaging for different applications, e.g ImageLab (Biorad) has pre-made program for pre-exposure with UV before imaging. The screenshot of using ImageLab to select the preset protocol to Image GlowPage in these instruments is shown below

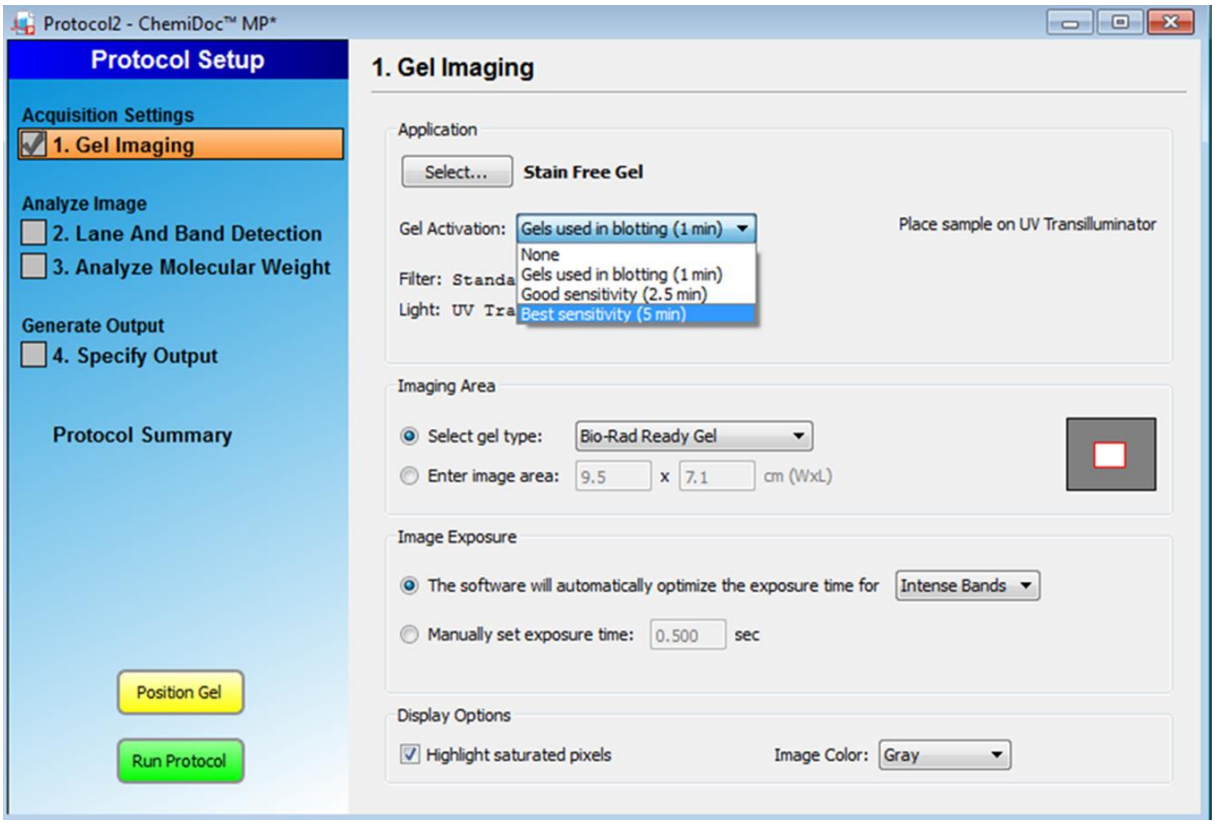
5.3.1 Selecting the Pre-set protocol in ImageLab for imaging Gel

Go to Gel Imaging>Protein Gels>Stain Free Gel



5.3.2 Go to Gel Activation menu and select **1 min**, **2.5 min**, **5 min** activation time (UV exposure time) depending upon intended downstream application and sensitivity required.

Note: *2.5 min of Gel activation time will give good amount of sensitivity for routine work which can be increased further by increasing activation time to 5 min. If downstream application is western blotting then we recommend 1 min of activation time.*



5.3.3 Click Position Gel

5.3.4 Click on Run protocol.

5.3.5 Follow Gel Doc manufacturer instruction for using ImageLab for **Protein quantification** or third party opensource software (ImageJ) for protein quantification.

6.0 Blotting of GlowPage Gels

6.1 Tank Blotting (Using the Mini Trans-Blot® Cell from Biorad)

6.1.1 Reagent Preparation

6.1.1.1 1X Tank Blotting Buffer(or Wet Transfer Buffer)

Reagent	Weigh	Final Conc.
Tris base	3.03 g	25 mM
Glycine	14.4 g	192 mM
diH2O	500 ml	
Methanol	200 ml	20 %

Adjust volume to 1 L with Distilled Water. pH will range from 8.1 to 8.5 depending upon the quality of reagents.

6.1.2 Protocol

- Soak 2 pieces of filter paper (the same size as the gel), 2 foam pads, and nitrocellulose membranes in 1x transfer buffer until wet; if PVDF (Provide with kit) is used, activate the PVDF by soaking in 100% methanol briefly, then immerse it in transfer buffer
- Open the cassette and place in a tray filled with transfer buffer; place a foam pad on the black side of the cassette
- Place a piece of filter paper on top of the foam pad, then carefully place the gel on top of the filter paper; remove bubbles with a roller
- Carefully place the membrane on top of the gel; if possible, do not move the membrane after it is positioned, and roll out any air bubbles
- Place a second piece of filter paper on top of the membrane, remove bubbles with a roller, and place the second foam pad on top of the filter paper
- Close the cassette and insert into the tank (the black side of the cassette should face the black side of the central core)
- Insert frozen cooling unit
- If transferring more than one gel, repeat the above steps with a second cassette
- Add transfer buffer to the tank until the buffer level reaches the upper fill line
- Place the lid on the tank to complete assembly

6.1.3 Run transfer: Recommended Transfer Conditions

Method	Standard Condition	Rapid Condition
Tank blotting	100 V, 30–60 min	150 V, 15–30 min*

6.2 Semi-Dry Transfer Cell Protocol

Following is the protocol recommended for SemiDry transfer using the Trans-Blot® SD Semi-Dry Transfer Cell (Biorad) with our GlowTransblot Buffer

#Items needed

- High Current Power Supply with output specifications of **100-500 V**, **2.5-3 A** (e.g. **PowerPac Universal Power Supply, Biorad**)

6.2.1 Reagent preparation

1X GlowTransblot Buffer

GlowTransblot Buffer (5X)	20 ml
Distilled Water	65 ml
Absolute Ethanol	15 ml

6.2.2 Protocol

- Soak 2 pieces of precut Thick Blot Paper
- Activate the PVDF membrane by soaking in 100% methanol briefly (30 sec), then equilibrate it in transfer buffer for 5 min
- Place one piece of precut **Thick Blot Paper** on the anode side of the semi-dry apparatus
- Place membrane (PVDF or nitrocellulose) on top of the filter paper
- Roll out any air bubbles.
- Carefully place gel on top of the membrane
- Add small amount of Transfer buffer dropwise across the surface of gel
- Gently roll out air bubbles
- Place the **second piece** of Thick Blot paper on top side of the gel; roll out any bubbles that may have formed between the stacks
- Carefully place the cathode assembly onto the transfer stack and then place the safety cover back onto the unit

6.2.3 Run transfer: Recommended Transfer Conditions

Target Protein	**Run Conditions**
1-150 kDa	25 V, 5min
150-250 kDa	25 V, 7 min

6.2.4 Image gel or transfer membrane **(Transfer Image)** under UV light to check transfer efficiency as described in section 5.2

Note: *A Pre-UV exposure of 2-5 min is not needed at this step. Protein on PVDF transfer membrane can be immediately visualized under UV Light*

7.0 Probing with Antibodies

Probe the proteins with Antibodies on membrane using your protocols and finally acquire the **Blot Image** *with Chemiluminescent Imaging*.

8.0 Total Protein Normalization

8.1 Using Image J

1. Quantify protein lanes of **Transfer Image** and respective protein bands in **Blot Image** using **Image J** open source plugin. Follow common protocol used for densitometry analysis in Image J.

   Note: *Use* ***Rolling Ball/Disc Background Substraction*** *functionality available in Image J for background substraction*
2. Normalize the protein bands quantification values with respective protein lane quantification values, just by dividing their value with protein lane values.
3. Use the normalized protein quantification value to get a fold change value by comparing it with respective control group.

8.2 Using Image Lab (Biorad)

If **Transfer Image** and **Blot Image** are acquired using **Image Lab Software** (Biorad), then follow their protocol recommended for **Total protein normalization.**

Reference: https://www.youtube.com/watch?v=k1xg6jbX3mo

Ordering Information

Catalogue	Description
GlowPAGE System	
GPH-7.5P-20	**GlowPAGE Gels, 7.5%, For casting 20 mini Gels**
GPH-7.5P-40	**GlowPAGE Gels, 7.5%, For casting 40 mini Gels**
GPH-7.5P-80	**GlowPAGE Gels, 7.5%, For casting 80 mini Gels**
GPH-7.5P-150	**GlowPAGE Gels, 7.5%, For casting 150 mini Gels**
GPH-10P-20	**GlowPAGE Gels, 10%, For casting 20 mini Gels**
GPH-10P-40	**GlowPAGE Gels, 10%, For casting 40 mini Gels**
GPH-10P-80	**GlowPAGE Gels, 10%, For casting 80 mini Gels**
GPH-10P-150	**GlowPAGE Gels, 10%, For casting 150 mini Gels**
GPH-12P-20	**GlowPAGE Gels, 12%, For casting 20 mini Gels**
GPH-12P-40	**GlowPAGE Gels, 12%, For casting 40 mini Gels**
GPH-12P-80	**GlowPAGE Gels, 12%, For casting 80 mini Gels**
GPH-12P-150	**GlowPAGE Gels, 12%, For casting 150 mini Gels**
GTB-0520	**GlowTransblot Buffer, For transferring 20 gels**
GTB-0540	**GlowTransblot Buffer, For transferring 40 gels**
GTB-0580	**GlowTransblot Buffer, For transferring 80 gels**
QTB-05150	**GlowTransblot Buffer, For transferring 150 gels**
GPR-10004	**GlowProtein Mix, 4 ml**
GPR-10010	**GlowProtein Mix, 10 ml**
GPR-10015	**GlowProtein Mix, 15 ml**
PVMINI-2045	**Precut PVDF Membrane (Mini Gel Size), 40 Pieces 0.45 µM**
PVMINI-2020	**Precut PVDF Membrane (Mini Gel Size), 40 Pieces 0.2 µM**