Enzyvaa

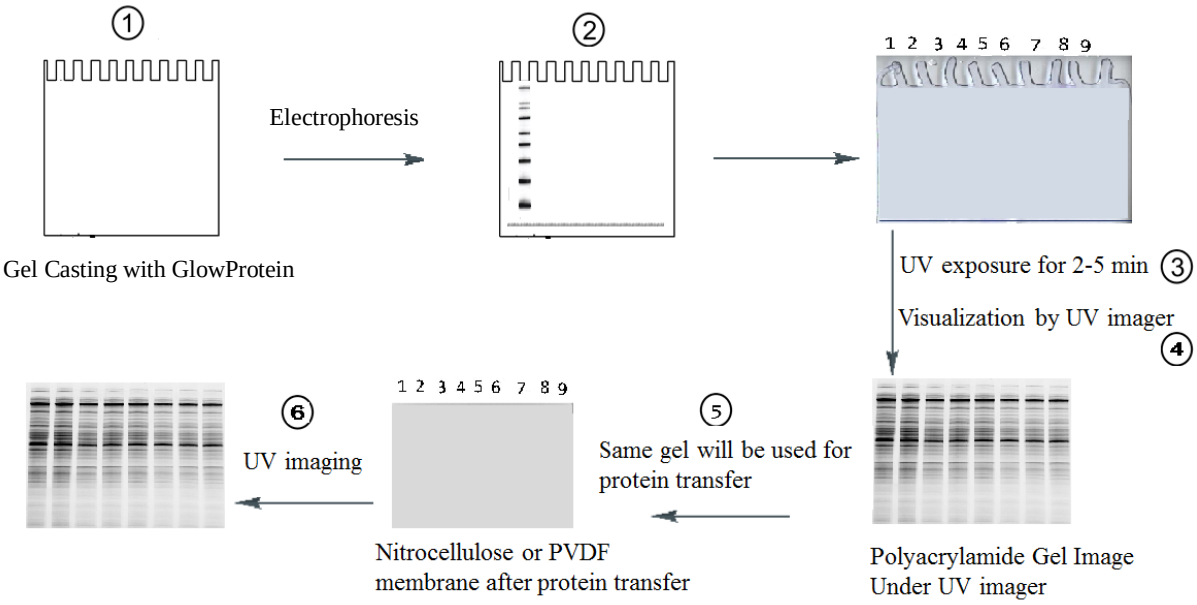
GlowProtein Mix

User Manual

Product description:

GlowProtein Mix provides an efficient protein detection system. It does not involve any fixation, staining or destaining. It is directly added in gel mix during the preparation of polyacrylamide gel. After completion of gel run, a short UV light induced photo-activation of protein in the presence of GlowProtein Mix makes the protein fluorescent. These fluorescent protein bands can easily be detected in polyacrylamide gel by UV imager (Gel Doc). Importantly, same polyacrylamide gel can be directly used for protein transfer on Nitrocellulose or PVDF membrane after visualisation of protein bands. Photo-activated protein retains their fluorescent property even after protein transfer on membrane. Hence, the protein bands can also be detected on nitrocellulose or PVDF membrane under UV exposure in Gel Doc system. Protein bands can be visualised multiple times without any additional step or negative effect on downstream applications. The photochemical activation of protein does not affect their immune detection properties, mobility during electrophoresis, and rate of protein transfer. Therefore, **GlowProtein Mix** is fully compatible with downstream applications such as western blotting, Mass spectroscopy and 2-D electrophoresis. The system is also compatible with SDS or native polyacrylamide gel irrespective of buffer composition and gel percentage.

Staining Workflow:



Protocol outline:

GlowProtein Mix based protein detection involves UV induced binding of **GlowProtein Mix** with protein, which generates fluorescence when exposed to UV light. Following are the basic steps involved:-

1. Addition of **GlowProtein Mix** during gel casting.
2. Electrophoresis of protein in polyacrylamide gel
3. Photo-activation of polyacrylamide gel under UV light (2-5min)
4. Direct UV imaging of acrylamide Gel or transfer membrane.

Detailed Protocol:

1. Addition of GlowProtein Mix during Resolving gel casting:

GlowProtein Mix is provided in 100x concentration, which needs to be diluted to 1X final concentration in resolving gel casting solution, i.e. 50 µl of GlowProtein Mix needs to be added in 5 ml gel casting solution.

Note : GlowProtein Mix needs to be added in resolving gel only*. No need to add it in stacking gel.*

Here is an example of routine polyacrylamide resolving gel casting scheme where GlowProtein Mix need to be added just during the gel preparation

	Gel Percentage				
Component	**8%**	**9%**	**10%**	**11%**	**12%**
Distilled water (µl)	2248	2098	1948	1748	1598
GlowProtein Mix (µl)	50	50	50	50	50
30% Acrylamide stock (µl)	1350	1500	1650	1850	2000
1.5 M Tris-HCL, pH 8.8 (µl)	1250	1250	1250	1250	1250
10% SDS (µl)	50	50	50	50	50
10% Ammonium Persulphate(µl)	50	50	50	50	50
TEMED (µl)	5	5	5	5	5
Final Volume (µl)	5000	5000	5000	5000	5000

Note*: **GlowProtein Mix** is compatible with all types of gels and buffering systems. In these systems, you just need to replace 1.5M Tris-HCL, pH 8.8 (as shown in above recipe) with your buffer system of choice.*

2. **Photo-activation of protein:**
   The photo-activation of polyacrylamide gel facilitates the conjugation of **GlowProtein Mix** with proteins. Proteins once conjugated with **GlowProtein Mix** can be detected directly under UV light at any later stage, both in the acrylamide gel or after transferred to the membrane.

   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 GlowProtein Mix and thus results in decreasing the sensitivity of detection.*

   ii) Expose the polyacrylamide gel for **2-5 minute** under UV light to form Glowprotein mix- 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.***

3. **UV imaging**

   Imaging of Acrylamide 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.

   Imaging of Nitrocellulose or PVDF membrane:

   For detection of protein on PVDF or nitrocellulose membrane, 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.

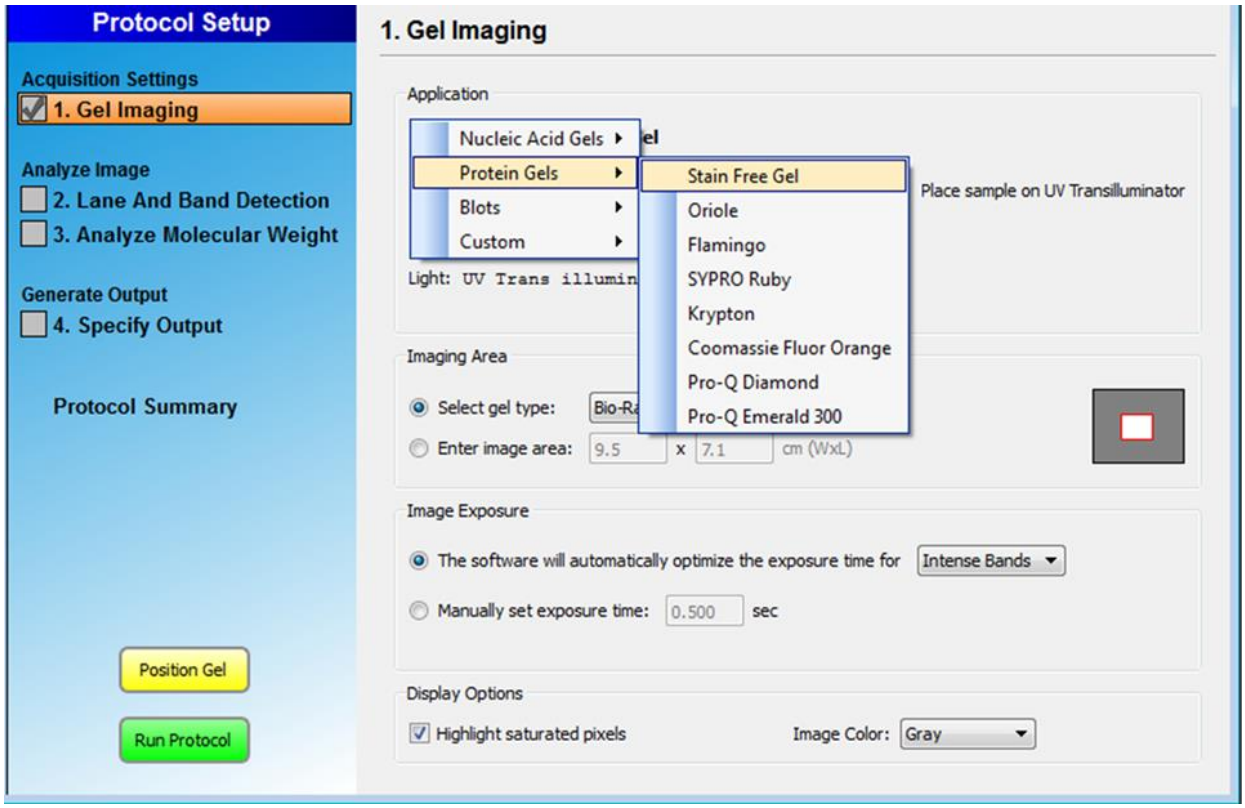
   Note: PVDF membrane should be used if you want to image the transfer membrane during western blotting. More preferably, PVDF membranes with low fluorescent background give very good results. We do not recommend use of internally supported nitrocellulose membrane for imaging protein bands on transfer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

Additional Information:

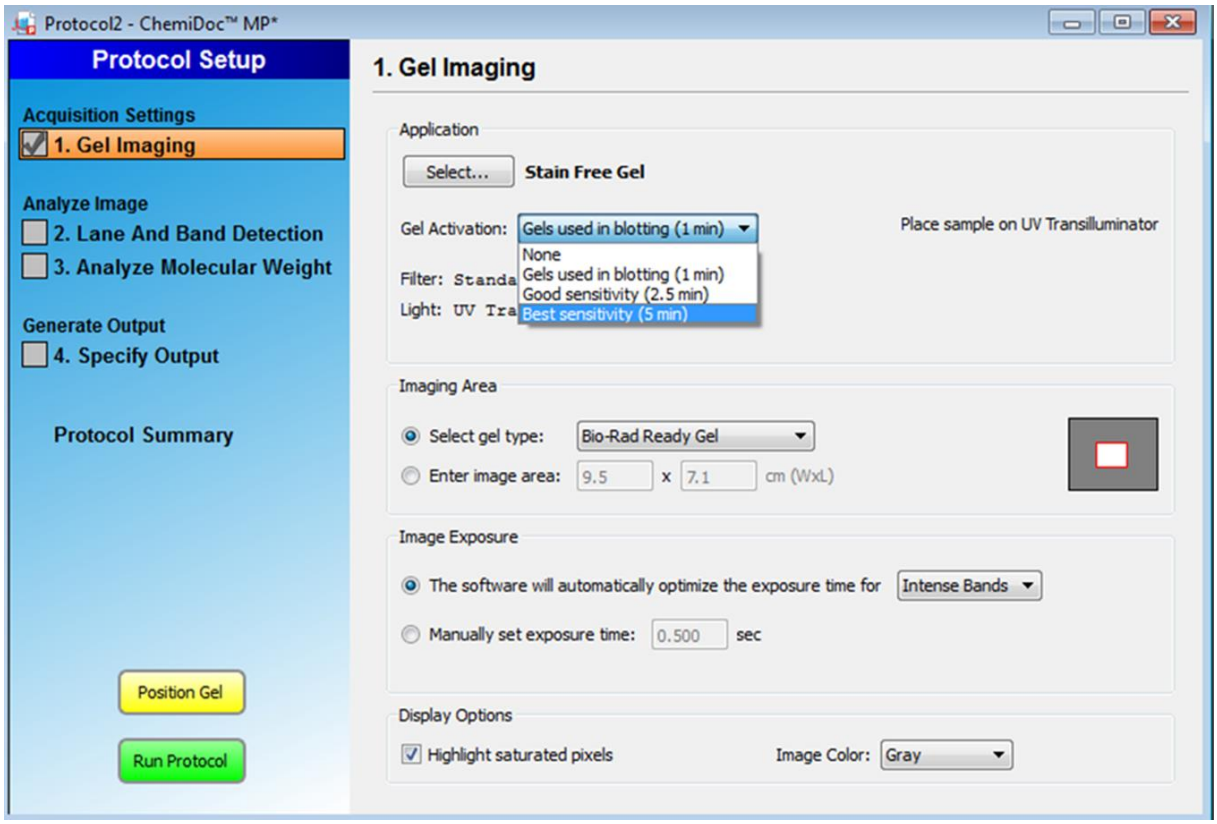
Some Gel Doc manufacturer such as Bio-Rad has pre-set protocol with pre UV exposure before imaging for different applications, e.g ImageLab (Biorad) has pre-made program for pre-exposure with UV before imaging. The screenshot of using ImageLab to select the preset protocol to use **GlowProtein Mix**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

1.Selecting the Pre-set protocol in ImageLab for imaging Gel

Go to Gel Imaging>Protein Gels>Stain Free Gel



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.



Step 3. Click Position Gel,

Step 4 Click on Run protocol.

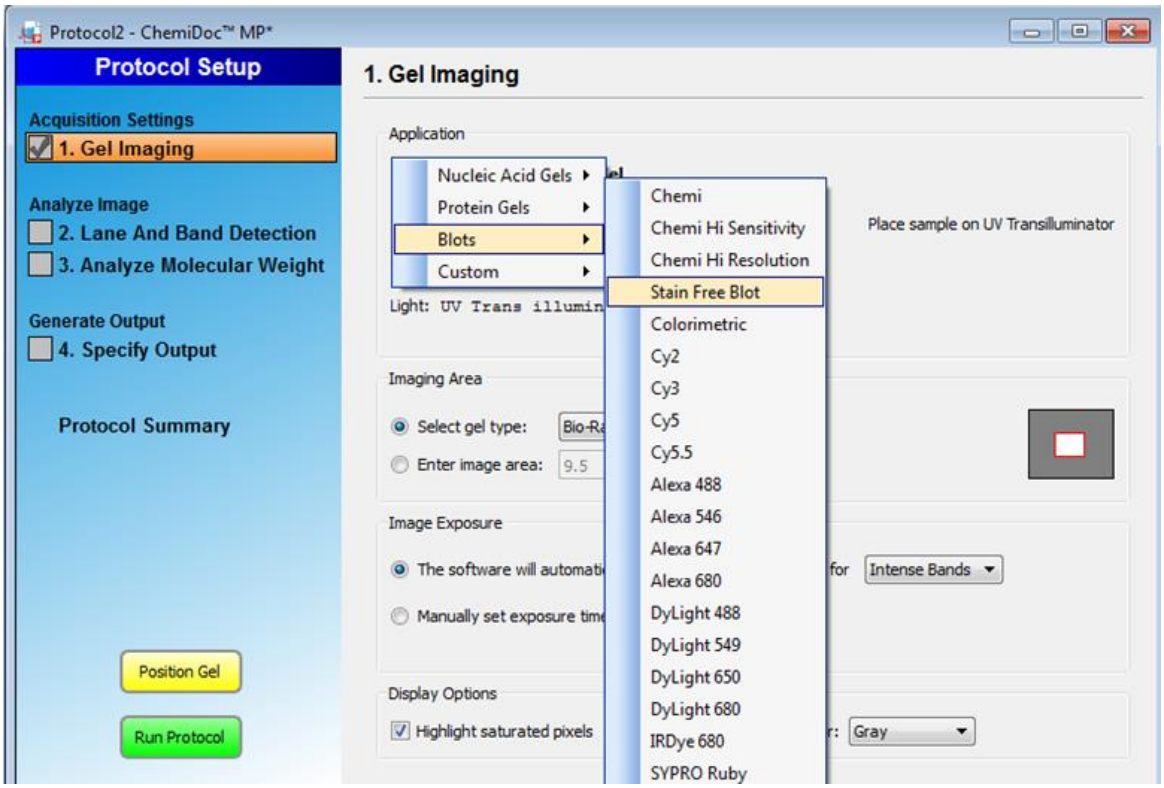
For detection of protein bands on transfer membranes

For detection of protein bands on transfer membrane using ImageLab software of Biorad Gel Doc System follow the instructions:-

Step 1) Go to Gel Imaging>Blots>Stain Free Blot

Step 2) Click Position Gel

Step 3) Click on Run protocol.



Sensitivity of detection:

The limit of detection of **GlowProtein Mix** is 8 to 28 ng depending upon amino acid content of proteins. The linear dynamic range of **GlowProtein Mix** is 10 to 80 µg of total protein from cell or tissue lysate.

Advantage of GlowProtein Mix:

Fast labelling and visualization:

GlowProtein Mix provides fast detection of protein bands without the requirement of any staining or destining procedure. Protein bands in the gel or transfer membrane can directly be visualized using Gel Doc. No additional steps for staining of polyacrylamide gel with coomassie or staining of transfer membrane with Ponceau S (or similar stains) are required

Compatible with downstream application: GlowProtein Mix is compatible with downstream applications such as western blotting, 2D electrophoresis and mass spectroscopy. Hence the same gel could be used for multiple applications after visualization, unlink the gel stained with coomassie blue, which cannot be used for downstream application such as western blotting.

Estimation of protein transfer efficiency: The efficient protein transfer from gel to membrane is critical for success of western blotting experiment. The GlowProtein Mix not only allow the detection of protein on nitrocellulose or PVDF membrane but also allow the validation of efficient transfer by comparing the protein band intensity in gel before and after transfer without the involvement of any staining or destaining procedure

Efficient normalization:

The basic requirement for housekeeping gene based normalization is that the expression of housekeeping gene should remain invariant under experimental condition. Hence, the band intensity of housekeeping in western blotting is representative of the total protein quantity loaded, and band intensity directly proportional to the amount of protein loaded. Therefore, the band intensity of target genes from different samples is normalized with housekeeping genes such as actin, β-tubulin, or GAPDH to eliminate the loading error introduced by protein qualification or by pipetting error.

An ideal reference gene should remain invariant under all experimental condition. But there is no universal housekeeping gene which remains invariant under all experiment treatments. Hence it is mandatory to validate the consistent expression of your housekeeping gene under particular experimental condition before using it as reference gene for normalization. Otherwise, it may introduce significant errors in the data.

Large number of recent studies have shown significant variation in the expression of different housekeeping genes under various experiment treatments or diseases including, cancer, diabetes, viral or bacterial infection. Hence, Normalization error forced various authors to issue errata or retraction of publications.

The oversaturated band of housekeeping gene in western blotting also loses the linearity with the total protein loading, which also introduced unexpected error in final data.

Therefore journals are now issuing strict guidelines for data normalization including the validation of consistency in housekeeping gene and with the experimental

condition(see guidelines from Nature Publication Group 2013 and guidelines from Journal of Biological Chemistry).

GlowProtein Mix offer whole protein based normalization where the total intensity of bands in each lane is normalized against another samples using software based background subtraction. The whole protein based normalization is superior than single gene based normalization and encouraged in recent guidelines for publishing on western blotting data. This also eliminates the use of housekeeping based normalization including the need of stripping and reprobing of housekeeping genes. Therefore, the method save time and improve the overall quality and reproducibility of data. The small difference between the expressions of target genes can also be detected by GlowProtein Mix based normalization. Ponceau S based whole protein normalization is also used sometime but band intensity of Ponceau S is weaker, and also the ephemeral nature of Ponceau S biding makes the quantification harder. Therefore GlowProtein Mix is a reliable, cost effective and more sensitive alternative of housekeeping based data normalization.

Only problem that you may face while using GlowProtein Mix for western blot normalisation is lane to lane inconsistency in traditional Laemmli based SDS-PAGE which decreases the quality of data if this inconsistency is severe. Therefore, we recommend our **GlowPAGE handcasting gels** for a consistent and reliable whole protein normalisation using **GlowProtein Mix.**

Related Products:

GlowPage Hand-Casting Gels: Allow easy casting, fast running (**20 min**), Whole protein Normalisation

QuickTransblot Buffer: Western blot transfer in 5 min with ~100 % efficiency

Heat Shock Free Competent Cell Maker: Make competent Cells that does not require any Heat Shock for transformation