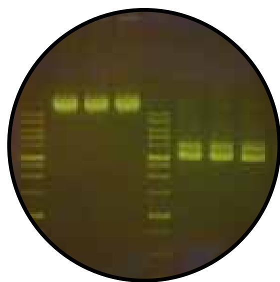
ProteinGOLD Fluorescent Total Protein Stain



Part Number:	PG-0010
Version:	1.0
Storage:	The product is stable for at least 6 months when stored at 2-8°C. Avoid exposure to temperatures greater than 37°C and protect from light.
Batch Number:	Marked on tube

GENERAL DESCRIPTION

ProteinGOLD is a fast and sensitive fluorescent dye for visualization and quantitation of proteins separated by 1-D or 2-D SDS-PAGE. It comes as a 100x stock solution that is simply diluted with water by the user to its working concentration. ProteinGOLD is normally low fluorescent but emits strong fluorescence (bright golden color) as bound to proteins. The staining procedure is a simple two-step protocol (fix and stain) that can be completed in as little as 30 minutes. Gels to be stained are fixed with ethanol/acetic acid solution prior to staining with ProteinGOLD solution. A destain step is not normally recommended, but may be employed to reduce background, simply by agitating the gel in water for 1-5 minutes. Gels stained with ProteinGOLD fluorescent gel stain may be directly visualized with a variety of different UV-based fluorescence imaging systems. The maximum emission wavelength of protein-bound ProteinGOLD is near 570 nm. ProteinGOLD gives exceptional sensitivity and wide dynamic range for protein detection. The bound ProteinGOLD dye is easily removed from the protein by immersing the gel in sufficient water, thus it is well compatible with subsequent enzymatic digestion and mass spectrometry for proteomics applications. Stained gels may be stored in stain solution in the dark at 2-8°C; imaging sensitivity might be moderately enhanced after 4°C storage of the stained gel.

EQUIPMENT REQUIRED BUT NOT SUPPLIED

1. Staining containers—Glass trays are recommended.
2. Imaging equipment — Gels are best imaged using a UV-based fluorescence imager capable of excitation near 330 nm and 390 nm and detection near 570 nm.
3. Laboratory shaker or rocker.
4. Powder-free latex, vinyl, or nitrile gloves.

REAGENTS REQUIRED BUT NOT SUPPLIED

1.Acetic Acid, reagent grade.
2.Ethanol, reagent grade.
3.Filtered, distilled or deionized water.

SAFETY CONSIDERATIONS

ProteinGOLD fluorescent gel stain is a 100x stock of an organic dye. It contains flammable solvent in its concentrated form and is suggested be handled with care. The diluted working solution is minimally hazardous and non-flammable. However, the complete properties of the dye component have not been investigated. Gloves and goggles should be worn and general laboratory safety precautions should be followed while handling both the undiluted and diluted products.

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DISPOSAL CONSIDERATIONS

Laws governing the disposal of laboratory chemicals vary by region. Consult the MSDS and check local laws for the proper disposal guidelines.

FLUORESCENCE CHARACTERISTICS

ProteinGOLD fluorescent gel stain has its excitation peaks at 330 and 390 nm and emission maximum at 570 nm, making it compatible with UV-based imagers.

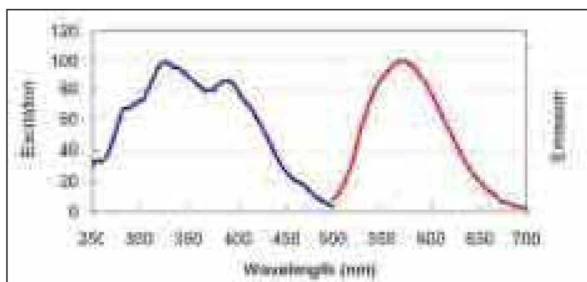

Figure 1. Excitation and emission spectra of ProteinGOLD.

TECHNICAL INFORMATION

Clean Technique

ProteinGOLD fluorescent gel stain is exceptionally fast and sensitive, Therefore, it is highly recommended to use clean technique to avoid dust or dirt transferred to the surface of the gel, which may appear in the fluorescence image as smudges or speckles. For best results, contaminant proteins such as keratins from skin and hair should also be minimized. All containers used should be well cleaned and rinsed with distilled or deionized water. Glass plates used to cast gels in the laboratory should be thoroughly cleaned with lint-free wipes. Use dust-free gloves and keep containers covered as much as possible.

Sensitivity of ProteinGOLD

ProteinGOLD fluorescent gel stain is highly sensitive, and the amount of proteins required to be visualized by ProteinGOLD is much less than what is possible by using conventional Coomassie Blue stain. Sensitivity of ProteinGOLD is of the same general order as silver stain or other fluorescent protein stains. The limit of sensitivity for individual proteins is around 1 ng or less.

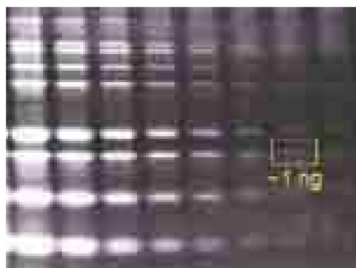

Figure 2. 1-D gel stained with ProteinGOLD.

Dynamic Range of ProteinGOLD

ProteinGOLD has a wide dynamic range with linearity covering three orders of magnitude. If needed, adjust the imaging conditions such as the exposure time to accommodate variability in the amount of protein to be visualized. As a general principle, the maximum quantity of protein recommended for visualization with ProteinGOLD is 1–2 μg for individual proteins and 10–20 μg for complex mixtures on 1-D gels.

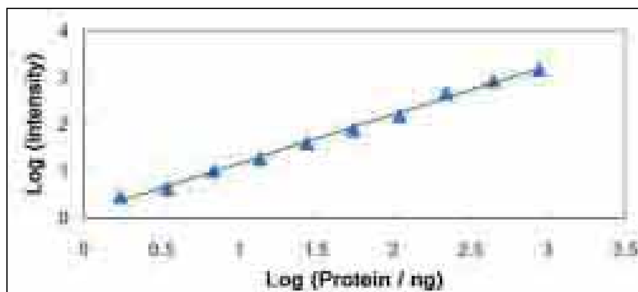

Figure 3. Linearity of ProteinGOLD covering three orders of magnitude.

Kinetics of ProteinGOLD

ProteinGOLD is fast to achieve saturation of protein binding at room temperature. Thirty minutes of incubation in ProteinGOLD is generally sufficient to obtain 50% saturation, and 45 minutes of staining at 25°C is close to saturation of detection. It's noted that stain at 4°C will delay the time to reach saturation but might enhance the sensitivity as compared with 25°C stain incubation.

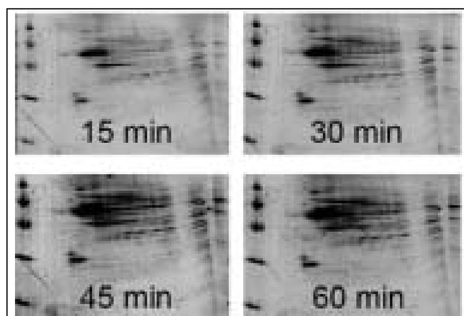

Figure 4. Fast detection of proteins on 2-D gel stained with ProteinGOLD at room temperature.

Gels Suitable for ProteinGOLD

ProteinGOLD is intended mainly for staining 1-D and 2-D SDS-PAGE for a variety of buffer systems and gel sizes. Instructions given here are for standard 1 mm thick gels. Thicker gels may benefit from longer stain times and larger volumes of stain solution.

Protein Markers Suitable for ProteinGOLD

Molecular weight standards that have been prestained with a visible dye do not stain with ProteinGOLD and thus cannot be imagined by fluorescence in gels stained with ProteinGOLD. We recommend additional use of unstained protein standards on gels for ProteinGOLD.

INSTRUCTIONS FOR USE OF PROTEINGOLD

Prepare Fix Solution

The fix solution consists of 40% (v/v) ethanol* and 7% (v/v) acetic acid. Prepare the solution with distilled or deionized water that has been filtered. The quantity of fix solution required depends on the number of gels to be stained and the size of the gel as indicated in the table below. As a general guide, use an amount of fix solution equal to 10-15 times the volume of the gel.

Gel Size	Volume of Fix Solution per Gel
~ 9 cm × 7 cm (general mini-gel)	~ 100 ml
~ 13 cm × 9 cm	~ 200 ml
~ 16 cm × 16 cm	~ 500 ml
~ 26 cm × 23 cm	~ 1,000 ml

* 40% ethanol can be replaced with 50% methanol, but methanol is not recommended in consideration of safety and stain performance.

Fix Gels

Remove the gel(s) from the gel cassette or plates. Place gel in a clean glass or plastic tray with the volume of fix solution indicated above. Cover the tray, place on a rocker or shaker and agitate gently.

For standard protocol, fix at room temperature for 60 minutes.

For quick fixing, microwave the fix solution with the gel to boil (~1.5 minutes for 100 ml of fixing solution; dependent on volume and microwave power), and then agitate gently at room temperature for 15 minutes.

Note: Either shortened or prolonged fix time may reduce sensitivity.

Prepare Working (1x) Stain Solution

Working (1x) stain solution is prepared by diluting one volume of ProteinGOLD with 100 volumes of distilled or deionized water that has been filtered. The quantity of stain solution required depends on the number of gels to be stained and the size of the gel as indicated in the table below. As a general guide, use an amount of working stain solution equal to 10 times the volume of the gel.

Gel Size	Volume of Fix Solution per Gel
~ 9 cm × 7 cm (general mini-gel)	~ 60 ml
~ 13 cm × 9 cm	~ 120 ml
~ 16 cm × 16 cm	~ 300 ml
~ 26 cm × 23 cm	~ 600 ml

Working (1x) stain solution is recommended to be freshly prepared prior to its intended use.

Stain Gels

Carefully pour off the fix solution and add ProteinGOLD (1x working solution) to the staining tray. Cover the tray, place on a rocker or shaker and agitate gently at room temperature.

For standard protocol, stain for at least 45 minutes

For rapid analysis, stain for 15-45 minute.

Generally, proteins (5 ng or below) might be detectable after 15 minutes of staining. Gels may be left in the ProteinGOLD solution for extended periods, with care to limit light exposure. Stained gels may be stored for up to three months without significant loss of fluorescent signals. For long-term storage, gels should be placed in sealable plastic bags with 5–10 ml of stain solution and stored in the dark at 2–8 °C.

NOTE: Destaining is not necessary. However, it might be helpful to remove excess dye from the gel surface by quickly rinsing the gel with clean water. Staining intensity persists or even increases when the gel is stored in ProteinGOLD solution at 4 °C.

Gel Imaging

Gels stained with ProteinGOLD are visualized using UV light excitation. We recommend to adjust excitation/ emission to around 330nm/570nm for best results. If the imaging equipment has no preprogrammed imaging function for ProteinGOLD, the imaging setting for SYPRO Ruby stain or ethidium bromide that uses UV transillumination is recommended. Any imaging system using UV light excitation may be used to image ProteinGOLD.

Subsequent analysis

ProteinGOLD fluorescent dye bound to proteins can be easily removed by keeping the gel in plenty of pure water or general buffered saline for hours. Analyses pertaining to imaging such as enzymatic digestion, mass spectrometry, and proteomics applications can be better conducted by use of dye-free proteins to minimize unwanted experimental interference from dye molecules attached.

Problem	Possible Cause	Remedy
Poor staining sensitivity	Too short or prolonged fixing period	Follow the recommendations for fixing.
	Too long destain/wash	Do not destain or wash the gel in water for more than 5 min. For long-term storage, keep the stained gel in stain solution at 4.
	Insufficient staining time	Stain sensitivity maximizes after 45 min.
	Dirty containers for staining	Make sure that the staining trays and other equipment have been thoroughly cleaned.
	Insufficient stain volume	Follow the recommendations for stain volume appropriate to the gel size.
	Reuse of the stain	Reuse of ProteinGOLD is not recommended.
High staining background	Excess dye remained on the gel surface	Quickly rinse the gel with clean water immediately before imaging. Staining sensitivity maximized after 30 min.
	Too much time in staining solution	Reduce time of staining if needed.
	Dirty equipment or staining trays	Make sure that the staining trays and other equipment have been thoroughly cleaned.
	Reagent impurities	Use high quality reagents for fix and stain solutions.
Speckles in the gel image	Particles from reagents and environment during operation	Ensure that the gel is prepared without particulate material and that staining trays are thoroughly cleaned. Use dust-free gloves and forceps to handle gels only by the edges. Limit exposure of gels and staining solution to open air.

Problem	Possible Cause	Remedy
Uneven staining	Insufficient shaking during staining	Make sure that the gel is well agitated and immersed during staining.
No detectable signals for protein bands or spots	Error of imaging system	Check instrument instruction or contact the manufacturer of imaging instrument.
	No protein on gel	Stain with Coomassie or silver stain to verify that there is actually protein on the gel

SIMPLIFIED PROTOCOL FOR ProteinGOLD

For one mini-gel

	Reagent	Standard Protocol	Rapid Protocol
Fix*	40% ethanol, 7% acetic acid	100 mL, 60 min once	100 mL, microwave to boil and then agitate at room temperature for 15 min
Stain	ProteinGOLD (1x working solution)	60 mL, 45 minutes or longer	60 mL, 15-45 minutes (30 minutes of staining might be sufficient for general applications)
Wash/Destain (optional)	Wash or destaining is not necessary for clear visualization. If needed, immerse the gel into 60 ml ddH2O with gentle agitation for 1-5 min. Note: Prolonged wash might largely reduce signals.		
Detection	Excitation: UV light (max: 330 nm and 390 nm) Emission: best at 570 nm, but also compatible with filters for EtBr stain and Sypro Ruby.		
Storage	Keep the stained gel in ProteinGOLD working solution at 4°C.		

* Note: Either shortened or prolonged fix time may reduce sensitivity.
** Use pure water for dilution to 1x working solution.