VISUAL PROTEIN

Manual

VisPRO™ 5 Minutes Protein Stain Kit

VP01-125/VP01-500/VP05-125/VP05-500

V2.0

Store at room temperature
For Research Use Only

Introduction

VisPRO™ 5 Minutes Protein Stain Kit (1 nanogram grade) provides a quick and easy way to read your protein gel. No fixation is required. Simply add the pre-made solution to your gel, and find the protein bands or spots in less than 5 minutes.

VisPRO™ 5 Minutes Protein Stain Kit applies the principle of zinc stain, a negative stain method. The formation of zinc-imidazole complex on polyacrylamide turns the gel to white, while the protein area prevents the stain and remains transparent. **VisPRO™ 5 Minutes Protein Stain Kit** perfectly matches to current proteomic research. Generally, more protein spots can be detected in 2-DE gels by this robust staining method than silver and Sypro Ruby stain. Moreover, the unfixing nature has it very ideal for subsequent analysis by mass spectrometry. Proteins developed by **VisPRO™ 5 Minutes Protein Stain Kit** usually have higher rate of good annotation.

VisPRO™ 5 Minutes Protein Stain Kit is also compatible to other techniques. The stained gel can be electro-eluted for recovering proteins, or electro-transferred onto PVDF or nylon membrane for Western blotting. The stained gel is also re-stainable by **VisPRO™ 5 Minutes Protein Stain Kit**, and all other known staining methods, such as Coomassie brilliant blue stain, silver stain and Sypro Ruby stain.

Product Components

VisPRO™ 5 Minutes Protein Stain Kit (VP01-125)

Solution 1 (Sensitizing Solution)	125 mL	1 bottle
Solution 2 (Developing Solution)	125 mL	1 bottle
User's manual		

VisPRO™ 5 Minutes Protein Stain Kit (VP01-500)

Solution 1 (Sensitizing Solution)	500 mL	1 bottle
Solution 2 (Developing Solution)	500 mL	1 bottle
User's manual		

VisPRO™ 5 Minutes Protein Stain Kit (5X) (VP05-125)

Solution 1 (5X) (Sensitizing Solution)	125 mL	1 bottle
Solution 2 (5X) (Developing Solution)	125 mL	1 bottle
User's manual		

VisPRO™ 5 Minutes Protein Stain Kit (5X) (VP05-500)

Solution 1 (5X) (Sensitizing Solution)	500 mL	1 bottle
Solution 2 (5X) (Developing Solution)	500 mL	1 bottle
User's manual		

Safety Information

Please wear gloves, lab coat and goggles while operating. Prevent contact product directly. In case of contacting, wash with large amount of water.

Storage

VisPRO™ 5 Minutes Protein Stain Kit could be stored at room temperature. Expiration date is labeled on the bottle or box. Shack the bottle gently before using.

Materials needed but not provided

1. Black Staing Box (BSB01/BSB02/BSB03, Visual Protein)
2. Gel Lighting Plate (GLP01, Visual Protein)
3. Flatbed scanner with transparency unit at transparent mode or CCD camera

Instruction

NOTE: Before use, dilute **Solution 1 (5X)** and **Solution 2 (5X)** to 1X with ddH_2O.

A. Quick Staining Protocol

1. After electrophoresis, move the gel into staining box with dark background. (Black staining box is available. BSB01/BSB02/BSB03)
2. Add Solution 1 to immerse the gel. Generally, 20 mL is sufficient for one 8 x 10 cm gel. Gently agitate the staining box (50-75 rpm) for 5 minutes.

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A. Quick Staining Protocol (~continued)

3. Discard Solution 1. Wash the gel briefly with ddH_2O for 5 seconds.
4. Add Solution 2 directly into the staining box.

> **NOTE:** Solution 2 "SHOULD NOT" be poured directly onto the gel surface. It will cause uneven background. Vigorously agitate the staining box by hand. The background of gel should turn white and the protein bands or spots should be visualized in less than 20 seconds.

5. Discard Solution 2 immediately. Wash the gel twice with ddH_2O.
6. Store the gel in ddH_2O. Depending on the quality of distilled water, the stained image can be sustained without protein diffusion from weeks to months.

> **NOTE:** For better storage, stained gel can be sandwiched between two transparency sheets and ziplocked in a plastic bag at 4 °C. In this way, gel images can be clearly discerned after months.It is possible to perform manual spot-picking from the stored gels.

B. Highly Sensitive Staining Protocol

1. After electrophoresis, move the gel into staining box with dark background. (Black staining box is available. BSB01/BSB02/BSB03)
2. Add SDS electrophoresis buffer (Tris/glycine buffer), 100 mL for one 8 x 10 cm gel. Gently agitate the staining box (50-75 rpm) for 5 minutes.
3. Discard SDS electrophoresis buffer.
4. Add Solution 1 to immerse the gel. Generally, 20 mL is sufficient for one 8 x 10 cm gel. Gently agitate the staining box (50-75 rpm) for 5 minutes.
5. Discard Solution 1. Wash the gel briefly with ddH_2O for 5 seconds.
6. Add Solution 2 directly into the staining box.

> **NOTE:** Solution 2 "SHOULD NOT" be poured directly onto the gel surface. It will cause uneven background. Vigorously agitate the staining box by hand. The background of gel should turn white and the protein bands or spots should be visualized in less than 20 seconds. "DO NOT" develop more than 1 Minutes, or it will cause overstaining.

7. Discard Solution 2 immediately. Wash the gel twice with ddH_2O.
8. Store the gel in ddH_2O. Depending on the quality of distilled water, the stained image can be sustained without protein diffusion from weeks to months.

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Important notification for staining 2-DE gels

NOTE: In some circumstance, the pH value and wetness of 2-DE gels are greatly changed during long time of electrophoresis. It is highly recommended to tuning your 2-DE gel conditions by submerge it in SDS electrophoresis buffer (Tris/glycine buffer) for 5-10 minutes before adding Solution 1. It is not required to wash the tuned gel before adding Solution 1.

NOTE: 20 mL, 50 mL and 100 mL of Solution 1/2 are sufficient to stain 8 x 10 cm,16 x 18 cm and 24 x 20 cm 2-DE gels, respectively. Because the 2-DE gels is often thicker, it is recommended to extend incubation of Solution I to 10-15 minutes for better staining results. It is recommended to cut the desired protein spots for the stained gel right after the staining.

C. Image acquisition

1. Scan the stained gel in the flatbed scanner with transparency unit at transparent mode. Turn on the option of reversing image. (recommend instrument)
2. When using CCD camera to document the gel image, a black background has to be place under the gel. However, the obtained gel image generally has less contrast.

D. Destaining and Restaining

- Destaining by SDS electrophoresis buffer (regular Tris/Glycine buffer with SDS)

1. The stained gel can be destained by SDS electrophoresis buffer in minutes.
2. If restaining of gel is required, just following the staining procedure starting from Solution 1.

- Destaining by 10% acetic acid

1. The stained gel can also be destained by 10% acetic acid in less than 1 minute. Fixation of protein will occur at the same time. 30 minutes fixation should be sufficient for complete immobilization of proteins.
2. If restaining of the acetic acid fixed gel is required, wash the gel three times by distilled water for 10 minutes. Equilibrate the gel in SDS electrophoresis buffer 5 minutes before restaining.

E. Gel visualization

1. The location of the protein on the stained gel can be easily observed by eyes when the gel is place on a black background, such as in the black staining box (BSB01/BSB02/BSB03).

E. Gel visualization (~continued)

2. When spot picking is required, it is recommended that to use Gel Lighting Plate (GLP01) for the best visual performance.

F. Other downstream applications

- Mass spectrometry

1. Move the cutting protein spots or bands into micro centrifuge tube (Eppendorf brand is recommended.)
2. Destained and fix the gels in 10% acetic acid for 30 minutes.
3. Wash the fixed gel thoroughly with three changes of ddH_2O.
4. The washed gel can then be stored at -20 °C or processed directly according to the standard mass sample preparation procedure.

- Electroelution / Electroblotting

1. Before electroelution or electroblotting is performed, Wash the gel 10 minutes by Tris/glycine buffer (without SDS).
2. Perform electroelution or electroblting according to standard procedures.

- Restaining by other staining methods

1. Fix the gel by 10% acetic acid for 10 minutes before performing Coomassie brilliant blue stain, silver stain, Sypro Ruby stain or other staining method.
2. Destain the gel by Tris/glycine buffer (without SDS) if activity stain is required.

Troubleshooting

Problem	Possible cause	Remedy
Background is too white and the contrast is poor	The gel may be over-developed	The optimal development by Solution 2 should be restricted to about 20-30 seconds
Background is not white and the contrast is poor	The pH of the original gel might be drifted (too acidic) or the original gel is too dry	Tuning your gel in SDS electrophoresis buffer before staining
Background turn white over 20 seconds	Most imidazole in the gel is removed by ddH_2O	Don't wash the gel with 100mL ddH_2O after discard Solution 1
Uneven background	Solution 2 is poured directly onto gel surface	Pour Solution 2 into area of staining boxes without gels
	The addition of Solution 2 is too slow	Add Solution 2 quickly
	The agitation of Solution 2 is not vigorous enough	Agitation vigorously when Solution 2 is added
	The contamination of protein from environments	Wear gloves. Clean all glassware and eletrophoretic apparatus.

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Appendix

Table 1. Comparison of staining methods

Method	VisPRO™ 5 Minutes Protein Stain	SYPRO™ Ruby	Silver Stain	CBR Stain
Preparation of solutions	0 min	5 min	20 min	0 min
Fixing step	0 min	1 hr	1 hr~overnight	30 min
Staining step	5 min	overnight	3 hr	30 min~overnight
Image display*	30 sec	30 min		30 min~overnight
Total time	5 min 30 sec	3-18 hr	4-20 hr	1-8 hr
Imaging instrument	Visble light	UV 302 nm or 480/620 nm	Visble light	Visble light
Irratated or toxic chemical	no	Acetic acid, Methanol	Acetic acid, Silver nitrate, Glutaraldehyde	Acetic acid, Methanol
Sensitivity	<1 ng	1 ng	1 ng	50 ng
Quantitative range**	1-200 ng	1-1,000 ng	1-80 ng	50-500 ng

* Including operating, developing and destaining time
** Quantitative might vary depending on the nature of proteins

Related Visual Protein Products

RIPA Cell Lysis Buffer (5X)	RP05-100	100 mL
Dual-Range™ BCA Protein Assay Kit	BC03-500	1 kit
Dual-Range™ Bradford Protein Assay Kit	BR05-500-K	1 kit
SDS-PAGE Running Buffer, 20 packs	RB500P	1 set
Gel Lighting Plate (210 x 297 mm)	GLP01	1 pc
Gel Lighting Plate (297 x 420 mm)	GLP02	1 pc
Black Staining Box (130 x 130 x 40 mm)	BSB01	1 pc
Black Staining Box (175 x 175 x 42 mm)	BSB02	1 pc
Black Staining Box (200 x 240 x 50 mm)	BSB03	1 pc
Black Staining Box (270 x 270 x 50 mm)	BSB04	1 pc