

Reverse Protein Stain Kit

For research use only

Sample	: protein electrophoresis gel
Format	: reagent
Operation time	: <10 minutes
Storage	: room temperature

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Introduction

Reverse Protein Stain Kit uses imidazole and zinc salts for protein detection (as low as 1 ng) in electrophoresis gels. The method is based on selective precipitation of a white imidazole–zinc complex in the gel, except in those zones where proteins are located. These zones remain transparent. When the gel is placed above a dark background, the negative gel image can be converted to a positive image with black bands, spots, and a white background. This stain is as sensitive as most silver stains and requires only up to 10 minutes to complete. In addition, fixation of proteins to the gel is not needed, there is no interaction of the stain with the protein, and complete destaining of the matrix can be achieved. Imidazole-zinc reverse stain uses only two staining solutions, which can be easily diluted from stock solutions. For the imidazole-zinc negative staining, no fixation is necessary. Gels are typically stained immediately after electrophoresis. Finally, imidazole-zinc reverse stain is fully compatible to down-stream applications, such as mass spectrometry, Edman sequencing, electroelution, and membrane blotting techniques.

Quality Control

The quality of the Reverse Protein Stain Kit is tested on a lot-to-lot basis by staining protein electrophoresis gel.

Kit Contents

Order Information

Name	PS050	PS500	Product Name	Package Size	Cat. No.
Protein Stain Buffer I	50 ml	500 ml	Prestained Protein Ladder IV	500 µl	PL004
Protein Stain Buffer II	50 ml	500 ml	Reverse Protein Stain Kit	500 ml	PS500
Black Stain Box	1 pc(s)	5 pc(s)	5X Reverse Protein Stain Kit	500 ml	PS505

Caution

Buffers contain harmful irritants. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References

Ortiz, M. L., Calero, M., Fernandez-Patron, C., Patron, C. F., Castellanos, L., and Mendez, E. (1992) FEBS Lett. 296, 300–304.

Additional requirements

ddH₂O

Reverse Protein Stain Kit Staining Protocol

Step 1	<ul style="list-style-type: none"> Following protein electrophoresis place the gel into the Black Stain Box. Wash gel in ddH₂O for a few seconds and discard the ddH₂O.
Step 2	<ul style="list-style-type: none"> Add enough Buffer I into the Black Stain Box to completely submerge the gel. Incubate gel in Buffer I for 5 minutes. During incubation shake the Black Stain Box vigorously by hand.
Step 3	<ul style="list-style-type: none"> Pour off Buffer I and wash gel in ddH₂O for 30 seconds 3-5 times and discard the ddH₂O. Add enough Buffer II into the Black Stain Box to completely submerge the gel (DO NOT pour Buffer II directly onto the gel surface). Incubate gel in Buffer II (During incubation shake the Black Stain Box vigorously by hand. DO NOT exceed 1 minute to avoid over staining). As soon as the background becomes white and the protein bands are visible pour off Buffer II immediately and wash gel in ddH₂O 3 times.
Step 4	<ul style="list-style-type: none"> Store the gel in ddH₂O

Reverse Protein Stain Kit Destaining Protocol

Step 1 SDS electrophoresis buffer destaining	<ul style="list-style-type: none"> Incubate gel in SDS electrophoresis buffer for 10 minutes. Begin with step 2 in the Staining Protocol if gel restaining is required.
Step 2 5% acetic acid destaining	<ul style="list-style-type: none"> Incubate gel in 5% acetic acid for 10 minutes. Wash gel with ddH₂O 3 times followed by gel equilibration using SDS electrophoresis buffer. Begin with step 2 in the Staining Protocol if gel restaining is required.

Troubleshooting

Problem	Possible Reasons/Solution
Background is too white and the contrast is poor	<p>Over developed gel</p> <ul style="list-style-type: none"> 20-30 seconds is optimal for development by Buffer II.
Background is not white and the contrast is poor	<p>The original gel pH might be too acidic or too dry</p> <ul style="list-style-type: none"> Prior to staining, gel should be tuned in SDS electrophoresis buffer.
Uneven background	<p>Buffer II is poured directly onto gel surface</p> <ul style="list-style-type: none"> Buffer II should only be poured into an area of the Black Stain Box which does not contain gel. <p>Buffer II addition is too slow</p> <ul style="list-style-type: none"> Add Buffer II quickly <p>Buffer II agitation is not vigorous enough</p> <ul style="list-style-type: none"> Following Buffer II addition, be sure and shake the Black Stain Box vigorously. <p>Protein becomes contaminated</p> <ul style="list-style-type: none"> Be sure and wear gloves and clean all apparatus thoroughly.
No protein band	<p>Protein gel is left in electrophoresis buffer for too long</p> <ul style="list-style-type: none"> Do not leave protein gel in electrophoresis buffer for extended periods such as overnight.