LIFE SCIENCE ANALYTICAL

TCI

Electrophoresis Reagents

Gel Staining Reagents

Silver Staining

New **Silver Stain Kit** [for Electrophorsis] 1kit [I1309]

Silver staining is a commonly-used method for the detection of proteins and DNA in polyacrylamide gels after electrophoresis. In this method, silver ions are bound to proteins and DNA present in the gel and reduced, resulting in stained bands. Silver staining is more sensitive than Coomassie Brilliant Blue (CBB) staining; it can detect down to nanogram amounts of protein.

Advantages

- **Rapid Staining (approx. 1 hour)**
- **Highly Sensitive (Several ng)**
- **Odorless - ammonium ion-free**
- **Safe - does not produce explosive silver amides**

Usage Example

1. Prepare Fixing Solution, Staining Solution, Developer Solution, and Stop Solution by diluting the supplied solutions 100-fold.
2. In a clean tray, submerge the gel in Fixing Solution, and allow to incubate with shaking for 10 minutes.
3. Remove Fixing Solution, and wash gel in deionized water with shaking for 10 minutes. (Repeat a total of three times)
4. Remove deionized water and replace with Staining Solution. Incubate with shaking for 5 minutes.
5. Remove Staining Solution and replace with deionized water. Incubate with shaking for 30 seconds.
6. Remove deionized water and replace with Developer Solution. Incubate with shaking for 30 seconds.
7. Replace old Developer Solution with fresh solution. Incubate with shaking until developed bands appear.
8. Remove Developer Solution and replace with Stop Solution. Incubate with shaking for 10 minutes.
9. Remove Stop Solution, and wash gel a total of three times with deionized water, incubating with shaking for 5 minutes each wash.

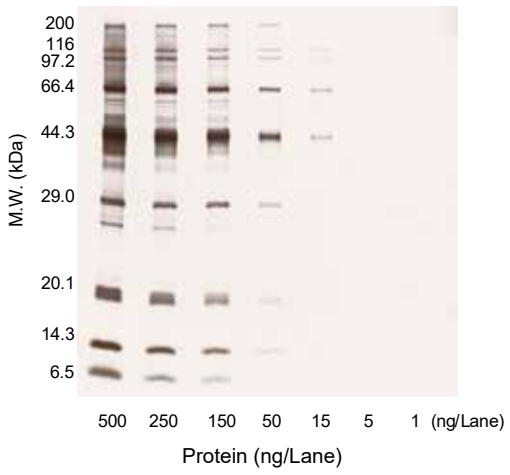


Figure A. Protein molecular weight markers were diluted, run on an acrylamide gel, and stained

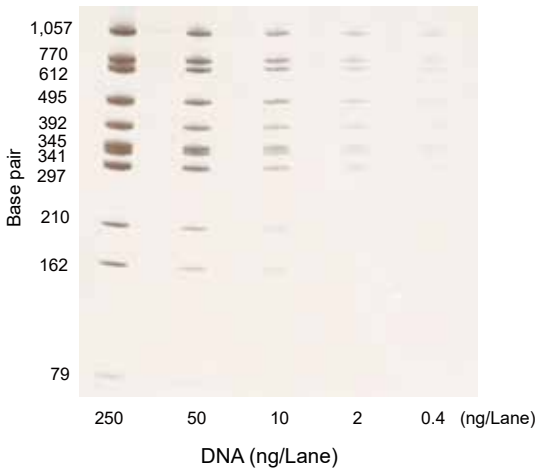


Figure B. DNA molecular weight markers at each concentration were diluted, run on an acrylamide gel, and stained

Negative Staining

Gel Negative Stain kit [for Electrophoresis] 1kit [G0615]

Negative staining is a detection method for SDS-PAGE-separated proteins in which only regions of gel not containing proteins are stained white, while protein-containing regions remain transparent. After staining, the gel can be easily destained with a destaining solution and transferred to a membrane.

Advantages

- **Rapid Staining (approx. 20 minutes)**
- **Highly Sensitive**
- **Allows the use of Destained Gels in Downstream Experiments**
- **Stains 20* Gels per Kit**
  ***90 x 90 x 1 mm gel**

Directions for Use

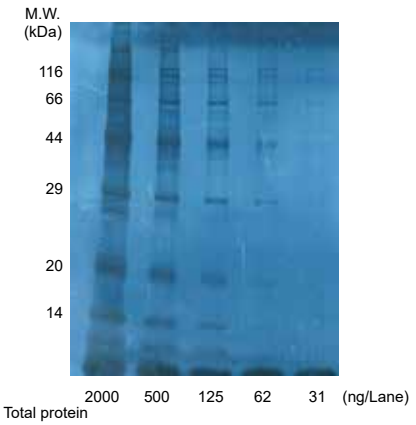
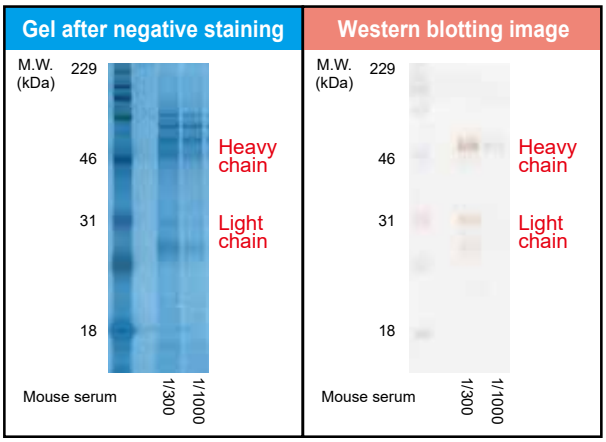


Figure. Gel stained by the method described at right.

1. Place the post-SDS-PAGE gel in a tray containing enough deionized water to cover the gel and shake for 10 minutes.
2. Discard the deionized water, add enough solution A (diluted 10 times with deionized water) to cover the gel and shake for 5 minutes.
3. Submerge gel in deionized water for 10 seconds to wash. Repeat three times.
4. Transfer the gel to a new tray, add enough solution B (diluted 10 times with deionized water) to cover the gel and shake for 1 minute to develop color.

Directions for Use (preparation of gel for Western Blotting)



1. Place the stained and photographed gel in a tray containing solution C diluted 10-fold with deionized water.
2. Shake the gel until the color is removed.
3. Discard solution C, add enough deionized water to cover the gel and wash for 30 seconds. Repeat three times.
4. Transfer the washed gel to a membrane (PVDF).

Primary Antibodies:
Goat Anti-Mouse IgG Biotin [G0387]
Secondary Antibodies:
Streptavidin HRP Conjugate [S0972]
Chromogenic Substrate:
3,3'-Diaminobenzidine (DAB) [D3756]

CBB Staining

Coomassie Brilliant Blue G-250 (Ready-to-use solution) [for Electrophoresis]
500mL [C3488]

Application

1. After electrophoresis, wash the gel with deionized water for 5 minutes three times.
2. Remove the water used for washing, add C3488 till the gel is soaked, and stain the gel for 1 hour while shaking gently at room temperature.
3. Remove the staining solution, destain the gel with deionized water for 1 hour and check it.
4. If the background is high, destain the gel with deionized water overnight at room temperature.

Figure. Proteins stained by the above method (destained overnight)

Comparison of Silver Staining, Negative Staining and CBB Staining

	Time	Detection sensitivity	Advantages
Silver Stain [I1309]	~1 hour	Several ng	Highly sensitive detection method with ample track record Able to detect both protein and DNA
Negative Stain [G0615]	15 - 30 minutes	Several ng	Short staining time Stained gels can be used for Western blotting, etc.
CBB Stain [C3488]	2 hours - over night	Several ng	Easy-to-use, simple protocol Resultant bands are quantifiable

Reagents for Protein Staining and Others

Acid Black 1 (= Amido Black 10B)	5g [A2097]
Acid Red 112 (= Ponceau S)	1g / 5g [A2256]
Coomassie Brilliant Blue G-250	5g [B3193]
Coomassie Brilliant Blue R-250	5g [B3194]
Fast Green FCF	5g [F0718]
Sodium Deoxycholate	25g [D1820]
6-Aminohexanoic Acid	5g / 25g [A2255]

Antibody Stripping Solution for Western Blotting

New Western Blot Stripping Buffer [for Biochemical Research] 250mL **[W0024]**

W0024 is used to strip antibodies from membranes that have undergone chemiluminescence detection. The antigen is retained on the membrane because the procedure is performed under mild conditions. This allows chemiluminescence detection to be repeated with a different antibody.

Example for use

1. Separate 2-fold step dilutions of Hela cell lysate (5.0 - 0.078 µg/lane) by SDS-PAGE.
2. After Western blotting, detect antibodies using chemiluminescence reagents (Figure A)
3. Immerse the membrane in TBS-T and shake for 10 minutes. Repeat this procedure twice.
4. Immerse the membrane in **W0024** and shake for 30 minutes at room temperature (Figure B).
5. Immerse the membrane in TBS-T and shake for 10 minutes. Repeat three times.
6. Start the blocking procedure again and detect the new antibody by chemiluminescence (Figure C).

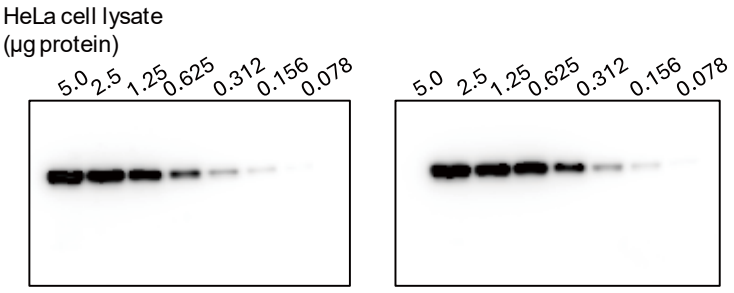


Figure A. First detection. Detection by binding of Anti-αTubulin Antibody (Rabbit IgG).

Membrane: PVDF membrane
Blocking Buffer: 1% BSA/TBS-T
Wash Buffer: TBS-T
Primary Antibody: Anti-αTubulin (Rabbit IgG)
Secondary Antibody: Goat anti-Rabbit IgG HRP
Detection: CCD Imager
Exposure time: 60 seconds

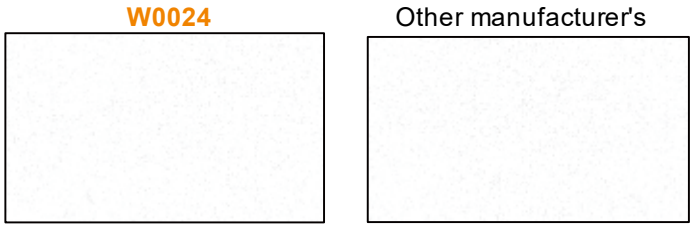


Figure B. Antibody removal with **W0024**

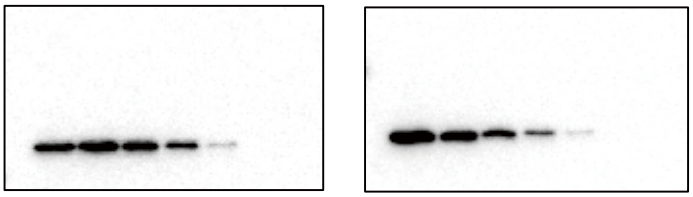


Figure C. Second detection. Detection by re-blocking the stripped membrane and binding Anti-GAPDH Antibody (Mouse IgG)

Blocking Buffer: 1% BSA/TBS-T
Wash Buffer: TBS-T
Primary Antibody: Anti-GAPDH (Mouse IgG)
Secondary Antibody: Goat Anti-Mouse IgG HRP
Detection: CCD Imager
Exposure time: 60 seconds

Chemiluminescent Reagent for Western Blotting

New **Chemiluminescence HRP Substrate Solution Kit** [for Western Blotting]
1kit [C4087]

C4087 is a chemiluminescent detection reagent for horseradish peroxidase (HRP)-labeled probes bound to proteins on membranes. The two-component type is used by mixing 1:1 immediately before use. Areas in the picogram range can be detected.

Example for use

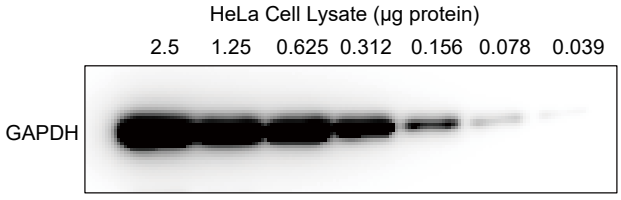


Figure. Membrane luminesced with C4087

HeLa Cell Lysate: 2.5 - 0.039 µg/lane (2x step dilution)
Membrane: PVDF membrane
Blocking Buffer: 1% BSA/TBS-T
Primary Antibody: Anti-GAPDH (Mouse IgG)
Secondary Antibody: Goat anti-Mouse IgG HRP
Detection: CCD Imager
Exposure Time: 120 seconds

1. Allow C4087 to reach room temperature.
2. Add HRP-conjugated antibody to the blotted membrane and wash.
3. Mix equal volumes of Solution A and Solution B.
   * Approximately 0.1 mL of mixture per 1 cm^2 of membrane.
4. Blot off excess wash buffer from the membrane.
5. Place the membrane on plastic wrap or a clear polyethylene sheet.
6. Pour the mixture over the membrane.
7. Allow to react for 60 seconds at room temperature.
8. Remove excess mixture.
9. Detect chemiluminescence.
   * Adjust exposure time according to signal intensity.

SDS-PAGE Sample Buffers

Sample buffers are available in three different concentrations to allow for easy use with any sample volume. No reducing agent is included - add as required. A red sample buffer is also available to help prevent sample mix-up.

2X SDS-PAGE Sample Buffer (2-Mercaptoethanol free) 25mL [B5834]
4X SDS-PAGE Sample Buffer (2-Mercaptoethanol free) 20mL [B6104]
6X Sample Buffer (2-Mercaptoethanol free) 10mL [B6105]
2X SDS-PAGE Sample Buffer Phenol Red (2-Mercaptoethanol free) 25mL [B6110]

Directions for Use

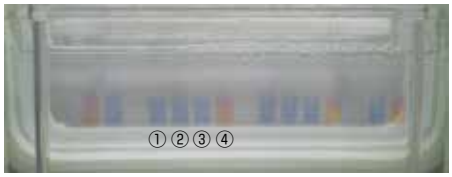


Figure. View during gel application.

Electrophoresis samples were prepared and applied to acrylamide gels using each of the following sample buffers.

① **2X SDS-PAGE Sample Buffer**
② **4X SDS-PAGE Sample Buffer**
③ **6X Sample Buffer**
④ **2X SDS-PAGE Sample Buffer Phenol Red**

Reagents for Gel Preparation, Buffer Preparation, etc.

30% Acrylamide / Bis-acrylamide (29:1)	250mL	[A3217]
30% Acrylamide / Bis-acrylamide (37.5:1)	250mL	[A3218]
Acrylamide Monomer	25g / 500g	[A1132]
Ammonium Peroxodisulfate	5g / 25g	[A2098]
Bromophenol Blue Sodium Salt (= BPB)	1g	[B3195]
DL-Dithiothreitol (= DL-DTT)	1g / 5g	[D3647]
Glycerol	1g	[G0316]
1-Thioglycerol	5g / 25g	[T3843]
Glycine	25g / 500g	[G0317]
2-Mercaptoethanol	5g / 25g	[M1948]
***N,N'*-Methylenebisacrylamide**	25g / 100g	[M0506]
Sodium Dodecyl Sulfate (= SDS)	25g / 500g	[S0588]
***N,N,N',N'*-Tetramethylethylenediamine (= TEMED)**	5g / 25g	[T2515]
Tris(hydroxymethyl)aminomethane (= Tris-Base)	25g / 500g	[T2516]

Related Reagents for Western Blotting

4-Chloro-1-naphthol (Ready-to-use solution) [for Western blotting]	100mL	[C3384]
Nitro Blue Tetrazolium / 5-Bromo-4-chloro-3-indolyl Phosphate *p*-Toluidine Salt Solution (50X) [for Western blotting]	5mL	[N1113]
TMB [for Western blotting] (Ready-to-use solution)	100mL	[T3855]
DAB staining kit	1kit	[D5909]
6-Aminohexanoic Acid	5g / 25g	[A2255]
Anti-6xHis Monoclonal Antibody (6A12) HRP Conjugate	1vial	[A3075]
Anti-Protein A Chicken Polyclonal Antibody HRP Conjugate	1vial	[A3187]
Anti-α Gal Chicken Polyclonal Antibody HRP Conjugate	1vial	[A3195]
Goat Anti-Mouse IgG HRP Conjugate	1vial	[G0407]
Goat Anti-Mouse IgM HRP Conjugate	1vial	[G0417]
Goat Anti-Rabbit IgG HRP Conjugate	1vial	[G0418]
Protein A HRP Conjugate	1vial	[P2466]
Streptavidin HRP Conjugate	1vial	[S0972]
Sheep Anti-Chicken IgY HRP Conjugate	1vial	[S0999]
Goat Anti-Mouse IgG FITC Conjugate	1vial	[G0406]
Goat Anti-Rabbit IgG FITC Conjugate	1vial	[G0452]
Goat Anti-Mouse IgM FITC Conjugate	1vial	[G0453]
Goat Anti-Mouse IgG DTBTA-Eu^{3+} Conjugate	1vial	[G0505]
Goat Anti-Rabbit IgG DTBTA-Eu^{3+} Conjugate	1vial	[G0506]
Goat Anti-Mouse IgG R-PE Conjugate	1vial	[G0569]
Goat Anti-Rabbit IgG R-PE Conjugate	1vial	[G0577]
Goat Anti-Mouse IgG1 Fab Fragment Cyanine3 Conjugate	1vial	[G0598]

Nucleic Acid Staining Reagents

Ethidium Bromide (0.5mg/mL in Water) (in Dropper Bottle) [for Electrophoresis]

10mL [E1363]

Each drop contains 20 µg of Ethidium Bromide, so you can easily adjust the solution as final concentration. Convenient and safe to use because of dropper bottle.

Application

After electrophoresis, dilute **E1363** (1 drop / 40 mL) to 0.5 µg/mL with water or running buffer and incubate the gel for 15 minutes If you have to decrease background fluorescence, wash the gel in water for 15 minutes. In use of electrophoresis buffer solution, Ethidium Bromide incorporated into nucleic acid and can visualize band immediately by using UV transilluminator.

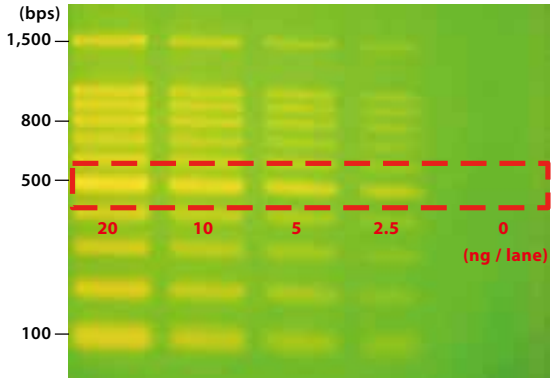


Figure. DNA Ladder Marker stained by the above method (destained 15 minutes)

10X Nucleic Acid Stain Blue [for Electrophoresis]

100mL [N1209]

Used to stain nucleic acids after agarose gel electrophoresis. As nucleic acids are stained blue, no transilluminator or other detection device is required. Unlike ethidium bromide, it is non-mutagenic and therefore safe and easy to handle.

Directions for Use

λDNA 200 100 50 25 12.5 (ng/Lane)

Figure. Gels stained as described on the right (after overnight washing).

1. Prepare **N1209** diluted 10 times with deionized water.
2. After electrophoresis, immerse the gel in the diluted **N1209** and shake for 10 minutes.
3. Discard the staining solution and shake the gel with deionized water for 30 minutes and wash the gel. Repeat the wash step if background is high.

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