SDS-PAGE Western Blotting





SDS-PAGE

CosmoPAGE Precast Gel

Features

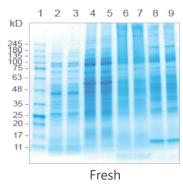
- Consistent performance
- · Compatible with Bio-Rad Mini-PROTEAN® Core
- Sharp, straight bands
- · Long shelf life; Up to 12 months

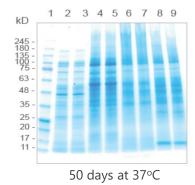




Consistent Performance

CosmoPAGE gels perform high protein resolution and reproducibility even stored at ambient temperature for 50 days.





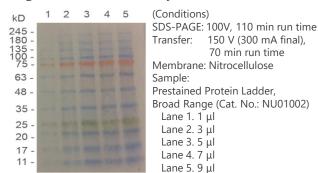
(Conditions)
Gel: CosmoPAGE TG Gel 4-15 %
Voltage: 150V for an hour
Sample:
Lane 1. Prestained Protein Ladder,
Broad Range, 3 µl
(#NU01002)
Lane 2. 3. E. coli lysate
Lane 4. 5. Hek293T lysate
Lane 6. 7. Mouse brain membrane
Lane 8. 9. Mouse brain cytosol
(10 µg each)

Compatibility

Compatible with the Bio-Rad Mini-PROTEAN® Core system without use of extra adapters.



High Transfer Efficiency



Protein Migration Chart

| | | TO | gel | Bis-Tris gel | | | | |
|-----------------|-----|----------------|---------------|---------------|--------------|----------------|-------------|--|
| | | 10 % | 10 % 4 -15 % | | 12 % | | 12 % | |
| 1 | 0 | TG | TG | MOPS | MES | MOPS | MES | |
| | 10 | 200 | | 200 | 200 | | | |
| l | 20 | 1000 | 200 | 116 | 116 66 | 200 | 200 | |
| <u>.</u> | | 116 | 116 | | — 4 5 | 116 | 116 | |
| % length of gel | 40 | 66 | | — 45 | — 31 | 110 | — 66 | |
| 듬 | 50 | | 66 | | 21 | 66 | — 45 | |
| ang | 30 | — 45 | | 31 | 14 | | — 31 | |
| <u>=</u> | 60 | | — 45 | 21 | — 6 | — 4 5 | 21 | |
| ĺ | /0 | — 31 | — 31 | | | — 31 | 14 | |
| Ī | 80 | | — 21 | 14 | | 21 | — 6 | |
| 1 | 90 | 21 | 14 | 6 | | 14 | | |
| + | 100 | | 6 | | | | | |



Ordering Information

| Product Name | Cat. No. | PKG size | Price |
|--|----------|----------|-----------|
| CosmoPAGE TG (Tris-Glycine) Precast Gel, 10%, 15 wells | NU001015 | 10 gels | \$ 89.00 |
| CosmoPAGE TG (Tris-Glycine) Precast Gel, 4-15%, 15 wells | NU041515 | 10 gels | \$ 89.00 |
| CosmoPAGE Bis-Tris Precast Gel, 12%, 15 wells | NU001215 | 10 gels | \$ 89.00 |
| CosmoPAGE Bis-Tris Precast Gel, 4-12%, 15 wells | NU041215 | 10 gels | \$ 89.00 |
| CosmoPAGE MOPS Running Buffer, Powder | NU01004 | 5 x 1 L | \$ 25.00 |
| CosmoPAGE Tris-Glycine Running Buffer, Powder | NU01011 | 5 x 1L | \$ 25.00 |
| Running Buffer Solution (10x) for SDS - PAGE, Tris - Glycine | 30329-61 | 1 L | \$ 63.00 |
| | 30329-74 | 5 L | \$ 231.00 |

Sample Buffer Solution for SDS-PAGE (6x)

Features

- · Suitable for low concentration protein sample adjustment
- · No precipitation in the refrigerator
- · Two types; with and without reducing agent

Comparison of 6x and 2x

The data right shows that both 2x and 6x sample buffer solution have the same separation patterns. Using the Sample Beffer Solution (6x) enables reduction of valuable protein sample for SDS-PAGE.

(Conditions)

Prepare two types of sample buffer solutions, A: 6x and B: 2x of protein markers and separated by SDS-PAGE with 12% acrylamide gel. For staining, CosmoPAGE Bullet CBB (NU 01003) was used.

Amount of protein of each lane was Lane 1: 5.1 μ g, 2: 2.5 μ g, 3: 1.3 μ g, 4: 0.51 μ g and 5: 0.26 μ g.

M.W. (kDa) 200 45 21.5 6x B 1 2 3 4 5

Ordering Information

| Product Name | Cat. No. | PKG size | Price |
|--|----------|----------|----------|
| Sample Buffer Solution with Reducing Reagent (6x) | 09499-14 | 5 ml | \$ 53.00 |
| Sample Buffer Solution without Reducing Reagent (6x) | 09500-64 | 5 ml | \$ 53.00 |
| Sample Buffer Solution without 2-ME (2x) | 30567-12 | 25 ml | \$ 59.00 |

Related Products

| Product Name | Cat. No. | PKG size | Price |
|--|----------|----------|-----------|
| T: (2 | 07277-61 | 1 g | \$ 91.00 |
| Tris(2-carboxyethyl)phosphine Hydrochloride [TCEP] | 07277-16 | 10 g | \$ 405.00 |

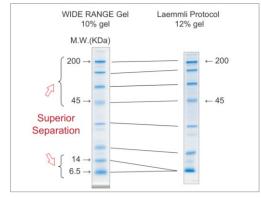


WIDE RANGE Gel Preparation Buffer (4x) for PAGE

Features

- Simple casting procedures
 WIDE RANGE Gel Preparation buffer is a 4x
 concentrated neutral pH buffer. It can be used for
 preparation of both stacking gel and resolving gel by
 replacing the Tris-HCL buffer in Laemmli buffer system.
- A wide separation range
 WIDE RANGE Gel provides a much greater separation range than the gel casted with a conventional Laemmli buffer system.
- Improved stability and strength
 The increased tensile strength allows easy handling even a low percentage gel. The neutral pH buffer improves the stability of gel resulting in a longer shelf life than the gel with Laemmli buffer system.

Comparison of Separation





Ordering Information

| Product Name | Cat. No. | PKG size | Price |
|---|----------|----------|-----------|
| WIDE RANGE Gel Preparation Buffer (4x) for PAGE | 07831-94 | 250 ml | \$ 103.00 |

Rapid Running Buffer Solution (20x)

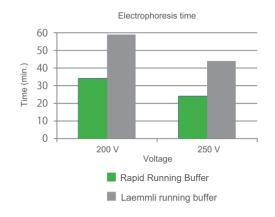
Features

- Fast Run Time: Running mini-gel in 25 minutes.
- Easy to Use: Premixed (20x) buffer just replace the Laemmli running buffer with Rapid Running buffer
- Wide-Range Separation: Gradient gel-like separation on a single percentage gel

Comparison of electrophoresis time



YouTube: Rapid Tunning Buffer



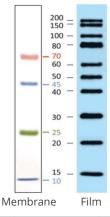
| Product Name | Cat. No. | PKG size | Price |
|-------------------------------------|----------|----------|----------|
| Rapid Running Buffer Solution (20x) | 12981-74 | 250 ml | \$ 58.00 |

Protein Ladder

CosmoPAGE WB Ladder

Features

- 4 prestained proteins for clear visualization during electrophoresis and Western blotting transfer
- 10 IgG-binding proteins for visualization on Western blots
- · Wide range size estimation (10-200 kDa)
- · Two enhanced bands (30 and 80 kDa)

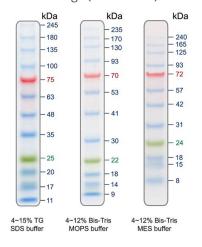


Ordering Information

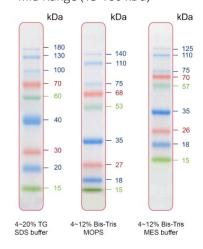
| Product Name | Cat. No. | PKG size | Price |
|---------------------|----------|----------|-----------|
| CosmoPAGE WB Ladder | NU01008 | 250 μΙ | \$ 160.00 |

Prestained / Unstained Protein Ladder

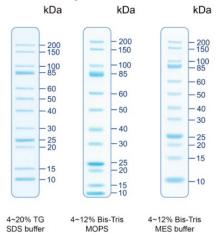
Cat. No.: NU01002 Prestained Protein Ladder, Broad Range (10-245 kDa)



Cat. No.: NU01009 Prestained Protein Ladder, Mid Range (15-180 kDa)



Cat. No.: NU01010 Unstained Protein Ladder, Broad Range (10-200 kDa)



| Product Name | Cat. No. | PKG size | Price |
|--|----------|----------|-----------|
| Prestained Protein Ladder, Broad Range | NU01002 | 500 μΙ | \$ 100.00 |
| Prestained Protein Ladder, Mid Range | NU01009 | 500 μΙ | \$ 100.00 |
| Unstained Protein Ladder, Broad Range | NU01010 | 500 μΙ | \$ 100.00 |

CosmoPAGE WB Ladder



Prestained Protein Ladder



Unstained Protein Ladder



Blocking Reagents

Bullet Blocking One

Features

- Fast blocking in 5 min.
- · Ready-to-use

Comparison Data

| Blocking Reagents | Bullet Blocking One | Blocking One | 5% Skim Milk |
|-------------------|---------------------|--------------|--------------|
| Blocking Time | 5 min. | 30 min. | 60 min. |
| Blotting Image | | | |

^{*} Blocking One is our standard blocking reagent.

(Conditions)

Blotting: Semi-dry Blotting Buffer Solution (Cat. No.: 30650) at 10 V for 30 min

Washing: 0.1% t-TBS (Cat. No.: 12750)
Blocking: Refer to the figure above

1st antibody: Anti-Vimentin (C-20) (Rabbit) (Cat. No.: SC-7557-R) diluted 1:2,000, 1 hr. at RT

2nd antibody: Anti-Rabbit IgG-HRP (Cat. No.: SC-2004) diluted 1:100,000, 1 hr. at RT

Detection: Chemi-Lumi One (Cat. No.: 11644), 5 min. reaction time

Detector: LAS-3000 (High mode), 15 min. exposure time

Sample: 20 µg of HeLa cell extract, 5 serial two-fold dilution series

Ordering Information

| Product Name | Cat. No. | PKG size | Price |
|--|----------|----------|-----------|
| Bullet Blocking One for Western Blotting (#13779-56 x 4 bottles) | 13779-14 | 200 ml | \$ 63.00 |
| Bullet Blocking One for Western Blotting | 13779-01 | 1 L | \$ 189.00 |

Related Products

| Product Name | Cat. No. | PKG size | Price |
|--|----------|----------|----------|
| Dia dia a One | 03953-66 | 100 ml | \$ 42.00 |
| Blocking One | 03953-95 | 500 ml | \$ 63.00 |
| Blocking One-P for Immunoassays with Phospho-specific Antibodies | 05999-84 | 200 ml | \$ 52.00 |



Antibody Diluent

Signal Enhancer HIKARI

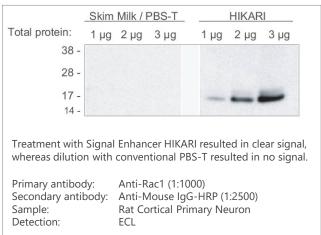
Dilute your antibodies with Signal Enhancer HIKARI instead of conventional diluents such as PBS-t or TBS-t before performing your next western blotting detection protocol and witness a remarkable increase in the ability to detect the protein of interest and to eliminate undesired background. Signal Enhancer HIKARI was developed to resolve the problems of low sensitivity and high background often encountered during procedures such as Western blotting and ELISA.

Features

- Enhances antigen-antibody reactions -
- · Removes background
- · Works with any substate
- · Works with any membrane
- · Ready-to-use reagent

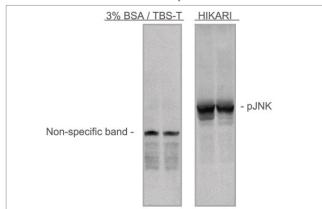
- yield several 10-fold increase in signal intensity
- improve the specificity of your antibodies
- enhances chemiluminescence, colorimetric and fluorescence detection system
- enhances signal from nitrocellulose and PVDF membrane
- just dilute your antibodies with Signal Enhancer HIKARI

Detection Enhancement of Rac1



Data courtesy of Dr. K. Ishizuka, Department of Psychiatry, Johns Hopkins University School of Medicine

Detection Enhancement of p-JNK



Treatment with Signal Enhancer HIKARI resulted in clear signal enhancement and background suppression compared with the conventional method using TBS-T.

Primary antibody: Anti-pJNK (1:1000)

Secondary antibody: Anti-Rabbit IgG-HRP (1:5000)
Sample: Mouse Embryonic Fibroblast (MEF)

Detection: Super Signal West Pico

Data courtesy of Dr. S. Matsuzawa, Signal Transduction, NCI Cancer Center, Burnham Institute for Medical Research

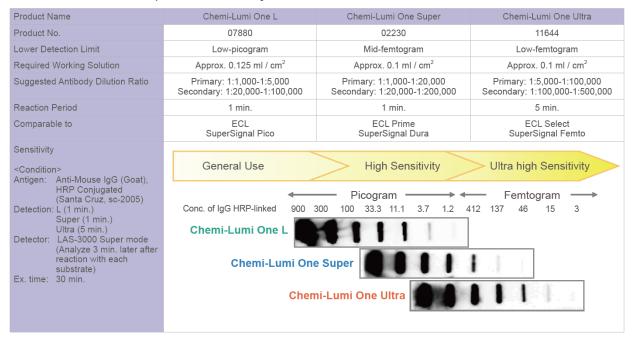
| Product Name | Cat. No. | PKG size | Price |
|--|-----------|----------|-----------|
| Signal Enhancer HIKARI 50 (Solution 1* 50 ml, Solution 2** 50 ml) | NU00101 | 1 kit | \$ 98.00 |
| Signal Enhancer HIKARI 250 (Solution 1* 250 ml, Solution 2** 250 ml) | NU00102 | 1 kit | \$ 298.00 |
| Signal Enhancer HIKARI - Solution 1 | NU00102-1 | 250 ml | \$ 179.00 |
| Signal Enhancer HIKARI - Solution 2 | NU00102-2 | 250 ml | \$ 179.00 |



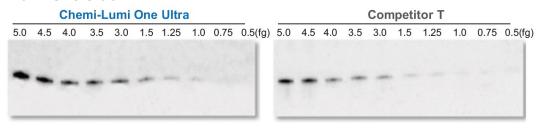
Chemiluminescent HRP Substrates

Chemi-Lumi One Series

Chemi-Lumi One is a series of highly sensitive luminol-based chemiluminescence assay kits for Western Blotting. Three types of chemiluminescent substrates are available for Western blot detection with horseradish peroxidase enzyme (HRP).



Chemi-Lumi One Ultra



Primary antibody: Rabbit anti-transferrin (1: 30,000) Secondary antibody: Goat-anti-rabbit IgG-HRP (1: 200,000)

| Product Name | Cat. No. | Quantity | PKG Size | Price |
|--|----------|-----------------------|----------|-----------|
| Chemi-Lumi One L (Luminol 50 mL, Peroxide 50 mL) | 07880-54 | 800 cm ² | 1 Kit | \$ 84.00 |
| Chemi-Lumi One L (Luminol 250 mL, Peroxide 250 mL) | 07880-70 | 4,000 cm ² | 1 Kit | \$ 200.00 |
| Chemi-Lumi One Super (Luminol 10 mL, Peroxide 10 mL) | 02230-14 | 200 cm ² | 1 Kit | \$ 84.00 |
| Chemi-Lumi One Super (Luminol 50 mL, Peroxide 50 mL) | 02230-30 | 1,000 cm ² | 1 Kit | \$ 210.00 |
| Chemi-Lumi One Ultra (Luminol 10 mL, Peroxide 10 mL) | 11644-24 | 200 cm ² | 1 Kit | \$ 116.00 |
| Chemi-Lumi One Ultra (Luminol 50 mL, Peroxide 50 mL) | 11644-40 | 1,000 cm ² | 1 Kit | \$ 328.00 |







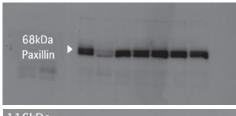
Stripping and Reprobing

WB Stripping Solution

Features

- No heating
- · No odor
- FastReady-to-use
- Reaction at room temperature
- Does not contain 2-mercaptoethanol
- Stripping time 5-15 minutes
- One solution in one bottle

Applications





First antigen-antibody reaction

Blocking: Blocking One (Cat. No.: 03953-95), 30 min

Wash: t-Tris Buffered Saline
Primary antibody: Anti-Paxillin (mouse IgG)
Secondary antibody: Anti-mouse IgG-POD

Detection: Chemiluminescence Detection Kit (commercially available product)

Stripping

RT, 15 min. for conventional protocol

Second, different antigen-antibody reaction

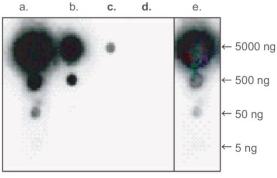
Blocking: Blocking One (Cat. No.: 03953-95), 30 min

Wash: t-Tris Buffered Saline

Primary antibody: Anti-Vinculin (mouse IgG) / Anti-Actin (mouse IgG)

Secondary antibody: Anti-mouse IgG-POD Detection: Chemi-Lumi One

Comparison of WB Stripping Solution and WB Stripping Solution Strong



Apply HPR-labeled anti-GST antibody to 5000 ng, 500 ng, 50 ng, or 5 ng (as desired) of c-Myc-GST antigen on a PVDF membrane, then remove the antibody by agitating gently for 10 minutes using one of the following stripping solutions.

a: 0.05%(v/v) t-TBS

b: 2%(w/v) SDS, 100mM 2-Mercaptoethanol

c: WB Stripping Solution

d: WB Stripping Solution Strong

After stripping the antibodies and washing the membrane with t-PBS for 2 min, use the chemiluminescence method to detect the HPR-labeled anti-GST antibody remaining on the membrane.

*Image "e" is a result that shows detection of the antigen with HPR-labeled anti-GST antibody on the "d" . The similar result is marked with "a" . Therefore, WB Stripping Solution Strong only stripped antibodies, not antigens.

| Product Name | Cat. No. | PKG size | Price |
|---|----------|------------|-----------|
| WB Stripping Solution | 05364-55 | 500 ml | \$ 98.00 |
| WB Stripping Solution Strong | 05677-65 | 500 ml | \$ 180.00 |
| WB Stripping Solution Trial Set (WB Stripping Solution + WB Stripping Solution Strong) | 05680-21 | 40 ml each | \$ 30.00 |

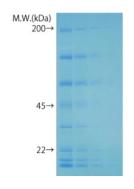


One-step Gel Staining Reagent

CosmoPAGE Bullet CBB

Features

- Fast Staining One-step staining in 15 minutes.
- Easy to Use No need for pretreatment, washing, destaining or microwaving.
- · Safe Use No harmful methanol or acetic acid is contained.





(Left)
15-min staining / No destaining
(Right)
Staining pattern in the CosmoPAGE Bullet CBB



Ordering Information

| Product Name | Cat. No. | PKG size | Price |
|----------------------|----------|----------|----------|
| CosmoPAGE Bullet CBB | NU01003 | 500 ml | \$ 98.00 |

Staining His-tagged Fusion Protiens

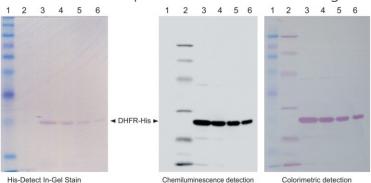
His-Detect In-Gel Stain

Features

- · Stain His-tagged fusion proteins directly in gels without western blotting
- · Detect picomolar level of His-tagged fusion proteins within an hour
- · No fluorescent image analyzer or transilluminator required



Application Data: Comparison with western blotting



Western blotting: Primary antibody: Anti-6xHis (Cat. No.: 04428) Secondary antibody: Anti-Mouse IgG-HRP (Goat) (SantaCruz, Cat. No.: SC-2005)

Cahemiluminescence detection: Chemi-Lumi One L (Cat. No.: 07880) Colorimetric detection: Peroxidase Stain Kit (Cat. No.: 26652)

* For detection of Protein Markers (Biotinylated), Streptavidin Horseradish Peroxidase Conjugate (Cat. No.: 02517) is used.

| Product Name | Cat. No. | PKG size | Price |
|-------------------------|----------|----------|-----------|
| | 08500-94 | 100 ml | \$ 50.00 |
| His-Detect In-Gel Stain | 08500-65 | 500 ml | \$ 189.00 |

Cell Lysis Solution

RIPA Buffer

RIPA Buffer (10x) is a ready-to-use solution containing a variety of surfactants and protease inhibitors (Cat. No.: 08714-04). Proteins lysed with RIPA Buffer can be used in western blotting, ELISA or immunoprecipitation testing regimes.

Features

- · Contains protease inhibitors (Cat. No.: 08714-04)
- Unmixes SDS solution for immunoprecipitation (Cat. No.: 08714-04)
- · Applicable BCA protein assay without buffer exchange

| Product Name | RIPA Buffer (1x, ready-to-use) | RIPA Buffer (10x) |
|-----------------------------|---|---|
| Protease Inhibitor Cocktail | without | with |
| Composition | - 50mmol/l Tris-HCl buffer (pH7.6) - 150mmol/l NaCl - 1% Nonidet® P40 - 0.5% Sodium Deoxycholate - 0.1% SDS | 【1x solution with SDS】 - 50mmol/I Tris-HCl buffer (pH7.6) - 150mmol/I NaCl - 1% Nonidet® P40 - 0.5% Sodium Deoxycholate - Protease Inhibitor Cocktail (1x) - (0.1% SDS) |
| Volume/Content | 100 mL | - RIPA Buffer(10x): 2ml×5本 - SDS Solution(1% SDS): 2ml×5本 |

Ordering Information

| Product Name | Cat. No. | PKG size | Price |
|-------------------|----------|----------|-----------|
| RIPA Buffer (10X) | 08714-04 | 1 set | \$ 136.00 |
| RIPA Buffer | 16488-34 | 100 ml | \$ 97.00 |

Inhibitor Cocktails

| Product Name | Cat. No. | PKG size | Price |
|---|----------|------------|-----------|
| Protease Inhibitor Cocktail Tablets, EDTA-Free | NU01069 | 20 tablets | \$ 325.00 |
| Protease Inhibitor Cocktail for Use with Mammalian Cell and | 25955-24 | 1 ml | \$ 63.00 |
| Tissue Extracts (100x) DMSO Solution | 25955-11 | 5 x 1 ml | \$ 208.00 |
| Phosphatase Inhibitor Cocktail | 07574-61 | 1 ml | \$ 136.00 |
| Phosphatase Inhibitor Cocktail (EDTA free) (100X) | 07575-51 | 1 ml | \$ 136.00 |





Protease Inhibitor Cocktail. Tablets



Protease Inhibitor Cocktail



Phosphatase Inhibitor Cocktail



TurboTEV Protease & Turbo3C Protease

Features

- · Activity over a broad temperature (4°C to 37°C) and pH (6.5 to 8.5) range
- · Both GST and His tags to facilitate its removal from the digested protein sample

Ordering Information

| Product Name | Cat. No. | PKG size | Price |
|--------------------------------------|----------|-----------------------|-------------|
| | NU0102S | 1,000 units (0.1 mg) | \$ 67.00 |
| TurboTEV (TEV Protease) 2 mg/ml | NU0102M | 10,000 units (1 mg) | \$ 480.00 |
| | NU0102L | 100,000 units (10 mg) | \$ 4,077.00 |
| TurboTEV (TEV Protease), HC 10 mg/ml | NU0112L | 1 x 10 mg | \$ 4,077.00 |
| | NU0101P | 100 units (0.1 mg) | \$ 42.00 |
| Turbo3C (HRV3C Protease) 2 mg/ml | NU010S | 1,000 units (1 mg) | \$ 338.00 |
| | NU0101M | 10,000 units (10 mg) | \$ 2,803.00 |

TurboTEV (TEV Protease)

Turbo3C (HRV3C Protease)





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