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Bullet PAGE Plus Precast Gel

1:	socratic gel		Gradient gel				
Product No.	Gel concentration	Wells	Product No.	Gel concentration	Wells		
21799-04	7 50/	13	21789-34	5 10%	13		
21800-54	7.5%	17	21790-94	5-10%	17		
21801-44	100/	13	21791-84	E 1E0/	13		
21806-94	10%	17	21792-74	5-15%	17		
21807-84	10 50/	13	21793-64	5 200/	13		
21811-14	12.5%	17	21794-54	5-20%	17		
21853-74	159/	13	21795-44	7 5 1 5 9/	13		
21854-64	15%	17	21796-34	7.5-15%	17		
			21797-24	10.20%	13		
			21798-14	10-20%	17		

*Refer to the reference data <Data 1> and <Data 2> for the separation pattern of each gel concentration.

Features

- Short SDS-PAGE running time: about 10 minutes
- Wide range of gel concentrations available
- Conventional Laemmli buffer system can be used for SDS-PAGE
- Surfactant-free, so available for nucleic acid analysis

Required equipment and reagents

- Electrophoresis Tank

Manufacture Name	Gellex International Itd.				
Product Name	WEP-MN Vertical Electrophoresis Tank				
Product No.	WEP-MN				

*If you are using a tank other than the one listed above, check the compatibility of the plate size of this product with it before use.

- Power supply

A power supply with the following performance is recommended to complete electrophoresis for two gels in about 10 minutes.

Output voltage	400 V or more
Output current	250 mA or more
Output power	100 W or more

*If you are using power supply other than the one listed above, the number of gel plates that can be run and the running time will differ. Please check the performance of power supply before use. For details on the performance of a power supply required, please refer to < Electrophoresis condition for one gel plate > in the protocol.

SDS-PAGE for Protein

- Sample buffer

Sample buffer (2x) composition

0.125 mol/L Tris-HCl, 4 (w/v)% SDS, 20 (v/v)% glycerin, 0.01 (w/v)% BPB, 10 (v/v)% 2-ME, pH6.8 <Related products>

Sample Buffer Solution with 2-ME (2x) for SDS-PAGE (Product No. 30566)

Sample Buffer Solution without 2-ME (2x) for SDS-PAGE (Product No. 30567)

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Sample Buffer Solution with Reducing Reagent (6x) for SDS-PAGE (Product No. 09499) Sample Buffer Solution without Reducing Reagent (6x) for SDS-PAGE (Product No. 09500)

*Protein sample preparation

For sample buffer (2x), mix protein sample and sample buffer at a volume ratio of 1:1. For sample buffer (6x), mix protein sample and sample buffer at a volume ratio of 5:1. After mixing, heat the diluted sample at 90° C for about 3 minutes.

- Running buffer

Running buffer (1x) composition

25 mmol/L Tris, 192 mmol/L glycine, 1 g/L SDS

<Related products>

Running Buffer Solution(10x) for SDS-PAGE (Product No. 30329)

Native PAGE for Nucleic acid

- Sample buffer

Loading Dye (6x) general composition 30 (v/v)% glycerin, 0.12 (w/v)% BPB, 0.12 (w/v)% XC, 0.6 (w/v)% Orange G, 50 mmol/L EDTA <Related products>

Loading Dye Brilliant Color (6x) (Product No. 11943)

*Nucleic acid sample preparation For Loading Dye Brilliant Color (6x), mix nucleic acid sample and Loading Dye at a volume ratio of 5:1.

Running buffer
 Running buffer (1x) composition
 25 mmol/L Tris, 192 mmol/L glycine
 <Related products>
 Running Buffer Solution(10x) for PAGE (Product No. 30340)

Protocol

1. SDS-PAGE for Protein

- 1. Take a gel plate from package.
- 2. As shown in Figure 1, hold the glass plate with both hands with the comb on the top, and slide the comb off while putting pressure inward with the thumbs.



3. Set the gel plate in a tank.

4. Carefully pour the running buffer into the tank, being sure to avoid trapping any air bubbles underneath the gel plate.

5. Load protein sample to wells.

6. After setting the electrophoresis conditions, proceed to run protein separation.

Electrophoresis condition for one gel plate> *For two gels, the current and power values are approximately do	Jpled
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Constant voltage	400 V	300 V	200 V
Maximum current	About 90-120 mA	About 70 mA	About 45 mA
Maximum power	About 36-48 W	About 21 W	About 9 W
Running time	About 10 mins.	About 16 mins.	About 30 mins.

*The running time for the electrophoresis may vary depending on factors such as the temperature of the buffer and the concentration of the gel.

*Refer to the reference data <Data 3> for the temperature variation of the Running buffer solution.

7. Stop protein electrophoresis.

<Guideline for ending protein electrophoresis>

When the BPB dye front is approximately 5 mm above the bottom edge of the gel (on the side with the positive electrode).

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8. Take the gel out of the tank, then insert a spatula between the glass plates, and use the principle of leverage to lift and remove the upper glass plate.

9. Using a spatula, carefully remove wells from the gel.

10. Transfer the gel to a tray filled with purified water or buffer solution, making sure that the gel is fully submerged.

2. Native PAGE for Nucleic acid

The protocol for this experiment is the same as the one outlined in section 1, 'SDS-PAGE for Protein'.

<Electrophoresis condition for one gel plate> *For two gels, the current and power values are approximately doubled.

Constant voltage	400 V	300 V		
Maximum current	About 80-100 mA	About 70 mA		
Maximum power	About 32-40 W	About 21 W		
Running time	About 12 mins.	About 18 mins.		

*The running time for the electrophoresis may vary depending on factors such as the temperature of the buffer and the concentration of the gel.

<Guideline for ending nucleic acid electrophoresis>

When the BPB dye front is approximately 5 mm above the bottom edge of the gel (on the side with the positive electrode).



Specification

Plate material	Glass				
Plate size	W100 mm × H80 mm × T3.2 mm				
Gel size	W80 mm × H60 mm × T1.0 mm				
Well number	13	17			
Well volume(/well)*	40 µL	28 µL			

Note: Contains 0.02% sodium azide.

*The recommended loading volume is below half the well volume. (13-well type: below 20 μL, 17-well type: below 14 μL)

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Attention

Packing

10 Sheets per package

- Run the electrophoresis at a constant voltage of no more than 400 V to avoid damage to your gel and apparatus.

- The recommended loading volume is below half the well volume. (13-well type: below 20 µL, 17-well type: below 14 µL)

If you notice distorted or skewed bands on your gel, this may be caused by high salt concentration in your sample.
 To fix this, you can purify your sample using the PAGE Clean Up Kit (Product No. 06441), or you can try loading your samples in a different order, such as leaving one lane open.

For more troubleshooting tips, consult the "Protocol for Electrophoresis" guide, which provides solutions for common electrophoresis problems.

If you're using the Protein Ladder One Plus, Triple-color for SDS-PAGE (Product No. 19593) in a high-concentration gel, you may notice that the shape of the low molecular weight band (10 kDa) is distorted by the composition itself.
 To fix this, try mixing the Protein Ladder One Plus with sample buffer and reducing the loading volume by half.
 For more information, refer to the reference data in <Data 4>.

Reference							
Laemmli, U.K : Nature, 227, 680 (1970)							
Storage							
Refrigerator (4-10 $^{\circ}$ C) (Do not freeze)							
Expiration							
Expiration date is stated on the product label.							

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Reference Data

<Data 1> Protein separation patterns at each gel concentration

Gel		Isocratic gel								
Gel %	7	7.5%	10%		12.5%		15%			
	M.W.(kDa)		M.W.(kDa)		M.W.(kDa)		M.W.(kDa)			
Electrophoresis Images (SDS-PAGE)	200 116 66 45 31 22+14+6.5		200 116 66 45 31 22 14 6.5		200 116 66 45 31 22 14 6.5		200 116 66 45 31 22 14 6.5			

Gel	Gradient gel										
Gel %	5-10	0%	5-15%		5-20%		7.5-15%		10	10-20%	
	M.W.(kDa)	M.'	M.W.(kDa)		M.W.(kDa)		M.W.(kDa)		M.W.(kD	a)	
Electrophoresis Images (SDS-PAGE)	200 116 66 45 31 14+6.5	2	200 116 66 45 31 22 14 6.5		200 116 66 45 31 22 14 6.5			200 116 66 45 31 22 6.5		200 116 66 45 31 22 14 6.5	

<Experimental conditions>

Sample:	Protein Markers(10x) for SDS-PAGE (Product No. 29458) prepared to 1 x, 3 µL added.
Running Buffer:	Running Buffer Solution(10x) for SDS-PAGE (Product No. 30329) prepared to 1x.
Electrophoresis:	Run at 400 V constant voltage and stop at the time When the BPB dye front is approximately 5 mm above
	the bottom edge of the gel.
Staining:	CBB Stain One (Ready To Use) (Product No. 04543)

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<Data 2> Nucleic acid separation patterns at each gel concentration



Gel	Gradient gel						
Gel %	5-10%	5-15%	5-20%	7.5-15%	10-20%		
Electrophoresis Images (PAGE)	$(bp) (1) (2) (3)$ $1500 \rightarrow \qquad \qquad$	$(bp) \textcircled{1} (2) \ \textcircled{3}$ $1500 \rightarrow \textcircled{1} (b) \ \textcircled{3}$ $1500 \rightarrow \textcircled{1} (b) \ \textcircled{3}$ $100 \rightarrow \textcircled{1} (b) \ \textcircled{3}$ $20 \rightarrow \textcircled{1} (b) \ \textcircled{3}$	(bp) ① ② ③ 1500→ 500→ 100→ 20→	(bp) ① ② ③ 1500 500 100 20	(bp) ① ② ③ 1500 500 100 20→		

<Experimental conditions>

Sample:	\odot 100bp DNA Ladder One(Ready To Use) (Product No. 07908) 2 μ L added.
	$@$ 20bp DNA Ladder (Takara Bio Inc. Product No. 3409A) 2 μ L added (Mix with 6x Loading Buffer).
	\Im siRNA Ladder Marker (Takara Bio Inc. Product No. 3430) 2 μ L added (Mix with 6x Loading Buffer).
Running Buffer:	Running Buffer Solution(10x) for PAGE (Product No. 30340) prepared to 1x.
Electrophoresis:	Run at 400 V constant voltage and stop at the time When the BPB dye front is approximately 5 mm above
	the bottom edge of the gel.
Staining:	Dual Green Nucleic Acid Stain (Product No. 20599)



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<Data 3> Protein separation patterns at different voltages

Gel	Bullet PAGE Plus Precast Gel, 5-20%, 17wells						
Constant voltage	400 V	300 V	200V				
Maximum current	92 mA	71 mA	45 mA				
Temperature increase observed	Δ7 °C	∆5.5°C	Δ3 °C				
Electrophoresis Images (SDS-PAGE)	$M.W.(kDa)$ $200 \rightarrow$ $45 \rightarrow$ $22 \rightarrow$ $6.5 \rightarrow$	M.W.(kDa) $200 \rightarrow$ $45 \rightarrow$ $22 \rightarrow$ $6.5 \rightarrow$	M.W.(kDa) $200 \rightarrow$ $45 \rightarrow$ $22 \rightarrow$ $6.5 \rightarrow$				

<Experimental conditions>

Sample:Protein Markers(10x) for SDS-PAGE (Product No. 29458) prepared to 1 x, 5 μL added.Running Buffer:Running Buffer Solution(10x) for SDS-PAGE (Product No. 30329) prepared to 1x.Electrophoresis:Stop at the time When the BPB dye front is approximately 5 mm above the bottom edge of the gel.Staining:CBB Stain One (Ready To Use) (Product No. 04543)

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<Data 4> Improvement of small molecule band shape in Protein Ladder One Plus

If you're using the Protein Ladder One Plus, Triple-color for SDS-PAGE (Product No. 19593) in a high-concentration gel (12.5%, 15%, 5-20%, 10-20%), you may notice that the shape of the low molecular weight band (10 kDa) is distorted by the composition itself. To fix this, mix Protein Ladder One Plus with sample buffer, and then load it to a well without heating.

In addition, when Protein Ladder One Plus 5 µL equivalent (10 µL or 6 µL added) is loaded, vertical streaks appear in the band, so reducing the amount added by half is recommended.

	Gel	Bullet PAGE Plus Precast Gel, 15%, 17wells								
Sample buffer		-	2X, with 2-ME (Product No. 30566)		2X, without 2-ME (Product No. 30567)		6X, with Reductant (Product No. 09499)		6X, without Reductant (Product No. 09500)	
Mix volume	Protein Ladder One Plus	5 µL	5 µL	2.5 µL	5 µL	2.5 µL	5 µL	2.5 µL	5 µL	2.5 µL
	Sample buffer	-	5 µL	2.5 µL	5 µL	2.5 µL	1 µL	0.5 µL	1 µL	0.5 µL
Loading volume		5 µL	10 µL	5 µL	10 µL	5 µL	6 µL	3 µL	6 µL	3 µL
Ele	ctrophoresis Images									

<Experimental conditions>

Sample: Protein Ladder One Plus, Triple-color for SDS-PAGE (Product No. 19593) prepared as followed.
 For sample buffer (2x), mix Protein Ladder One Plus and sample buffer at a volume ratio of 1:1.
 For sample buffer (6x), mix Protein Ladder One Plus and sample buffer at a volume ratio of 5:1.
 After mixing, load the amounts listed in the table to a well without heating.
 Running Buffer: Running Buffer Solution(10x) for SDS-PAGE (Product No. 30329) prepared to 1x.

Running Buffer: Electrophoresis:

Run at 400 V constant voltage and stop at the time When the BPB dye front is approximately 5 mm above the bottom edge of the gel.