For research use only

ISO9001

PeptiGel™ Peptide PAGE Analysis Kit

Product Name	Qty	Cat. No.	Remarkers
PeptiGel™ Peptide	1 Kit (net 200ml)	EBA-1053	

Description

PeptiGel™ Peptide PAGE Analysis Kit can be substituted for Tricine gel system to separate a small peptide (MW 2-30 kDa) in a polyacrylamide gel electrophoresis.

By using PeptiGel™ Peptide PAGE Analysis Kit, users can obtain highly resolved results without changing Tris-Gly-SDS buffer system. The minimizing heat generation during electrophoresis using PeptiGel™ Peptide PAGE Analysis Kit results in fast migration time and eliminating the smiling effect of bands commonly observed in Tricine gel system.

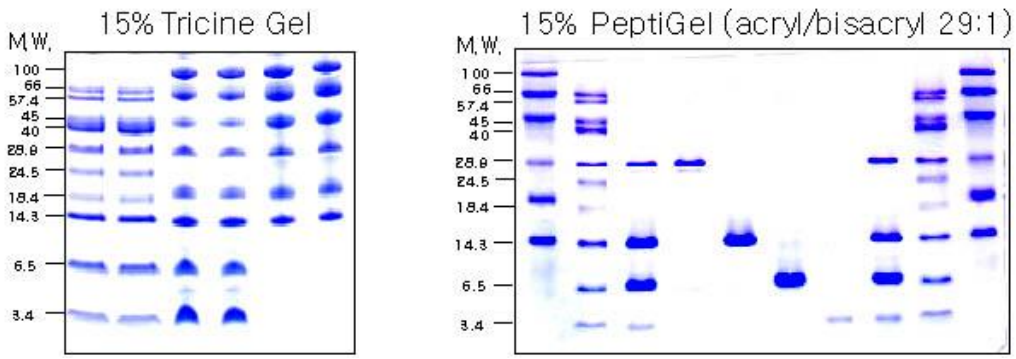
Kit contents

1. PeptiGel 2x Running Gel Buffer : 100 ml

The following materials are not included in the kit and should be prepared before making gel cast.
Acrylamide solution, 0.25 M Tris-Cl (pH6.8), TEMED, 10% (w:v) Ammonium persulfate, Protein sample buffer, Tris-Gly-SDS running buffer.

Storage Condition

Store at 4℃ for 1 year



Performance test of PeptiGel and conventional Tricine gel

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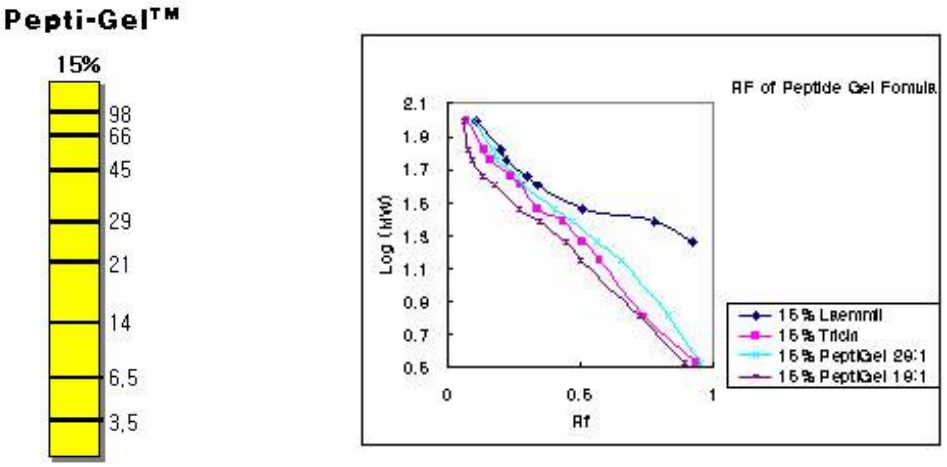

Migration pattern of PeptiGel and conventional Tricine gel

Protocol

1. Assemble the gel plates according to the manufacturer's instructions in the case of commercial apparatus.
   - In the case of small gel plate (8×10cm^2, 0.75 mm thick), 5 ml of running gel solution and 1 ml of stacking gel solution are required.

2. To make 10 ml of 15% running gel solution (29:1 or 19:1 acrylamide/bisacrylamide).

PeptiGel 2x running gel buffer	5 ml
40% Acrylamide/bisacrylamide gel stock (29:1 or 19:1)	3.75 ml
TEMED	5 µl
10%(w/v) Ammonium persulfate	0.1-0.2 ml
Add distilled water to final volume is 10 ml	

- There is no SDS in gel solution, but it is not important to separate and resolute proteins. In case of need, add SDS to final 0.1% concentration.

3. After the addition of TEMED and ammonium persulfate, transfer the mixed running gel solution into glass plate until indicated line previously. Gently fill the center of the glass chamber with the solution by allowing the solution to run down the side of one of the spacers. Be careful not to introduce air bubbles during this step.

4. Add 0.5 ml of distilled water or alcohol (ethanol or isopropanol) on top of the running gel solution. Done appropriately, the water will form a layer over the gel, and a clear line of demarcation will be observed as the gel polymerizes (at room temperature for 10-30min).

   - This keeps the gel surface flat. When the gel has polymerized, a distinct interface will appear between the running gel and the alcohol.

5. After polymerization is complete (around 30 minutes), rinse several times with distilled water.

Water remaining on the plates can be removed using pieces of filter paper.

For the stacking gel solution, mix the following:

0.25 M Tris-HCl (pH6.8)	1 ml
10% Acrylamide gel stock	1 ml
TEMED	2 µl
10% Ammonium persulfate	20-40 µl
Total volume	2 ml

6. Pour mixed staking gel solution onto running gel until solution reaches top of front plate, carefully insert comb and stacking gel to polymerize.

- Be careful not to trap any air bubbles beneath the combs. Oxygen inhibits polymerization, and will subsequently result in poor protein separations.

7. After the stacking gel polymerization is complete (around 10 minutes), remove the comb carefully and rinse wells several times with distilled water.

8. Remove the gel from its casting stand and assemble it into the appropriate electrophoresis chamber.

Fill the chamber with electrophoresis buffer (25 mM Tris, 192 mM glycine pH8.3, 0.1% SDS) to upper and lower buffer reservoir, making sure that well is immersed in buffer.

9. Combine protein sample and 1× SDS gel-loading buffer (50 mM Tris-Cl, pH 6.8, 1% β-mercaptoethanol, 2% SDS, 0.01% bromophenol blue, 10% glycerol). Load sample solutions into well carefully.

- You can use 0.01% phenol red instead of bromophenol blue as a tracking dye. Using phenol red may help you to increase resolution.

10. Assemble the top of the electrophoresis apparatus and connect the system to an appropriate power source. Be sure that the cathode (-) is connected to the upper buffer chamber. Ensure that the electrodes are correctly connected.

- In the case of small gel ($8 \times 10 cm^2$), electrophoresis has performed at constant voltage of **100-150 V**.

It may consume **2.5-3 hrs** to complete the electrophoresis.

- High voltage or current may result in generation of air bubbles in gels during electrophoresis affecting the resolution.