For research use only

ISO9001

Gradi-Gel™ II Gradient PAGE Analysis Kit

Product Name	Qty	Cat. No.	Remarkers
Gradi-Gel™ II Gradient PAGE analysis kit	1 Kit (50 mini gels)	EBA-1056	

Description

Gradi-Gel™ II Gradient PAGE analysis kit is a novel product of Elpis Biotech to make up for defects of conventional gradient gel preparation. Preparation of conventional gradient gel using a gradient former is a time consuming and tedious job itself. Furthermore, hand-made gradient gel is not reproducible in electrophoretic results and sometimes results in distorted images because of difference in polyacrylamide gel percentage between upper and lower part.

Elpis Biotech's Gradi-Gel™ II Gradient PAGE analysis kit uses single percentage polyacrylamide gel employing the chemical gradient formation during gel electrophoresis instead of mechanical gradient. Gradi-Gel™ II gradient PAGE analysis kit is composed of 2x running gel buffer and 2x stacking gel buffer. By simply mixing buffers and acrylamide solution, users can prepare a linear migrating gradient gel without unnecessary labor.

Gradi-Gel™ II Gradient running gel is overlaid with a lower percentage stacking gel, ensuring highly separating performance and provide you with highly resolved razor-sharp protein banding.

Gradi-Gel™ II Gradient PAGE analysis kit is completely compatible with conventional Tris-Glycine-SDS running buffer for denaturing PAGE, and Tris-Glycine for native and non-denaturing PAGE.

1. Convenient, ready-to-use, reproducible gradient polyacrylamide gels
2. High resolution (razor-sharp bands)
3. Fast running speed (1.5-2 hrs at 100 V constant voltage)
4. No need to buffer change (Tris-Glycine-SDS buffer system)
5. Small peptide analysis using low percentage gels
6. Optimized composition for western transfer and blotting, brilliant blue and silver staining of gels
7. No distortion and shrinking of dried gels (because of single % gradient gel)

Kit contents

1. Gradi-Gel™ II 2x Stacking Gel Buffer : 20 ml
2. Gradi-Gel™ II 2x Running Gel Buffer : 100 ml

Storage condition

Store at 4℃ for 1 year

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

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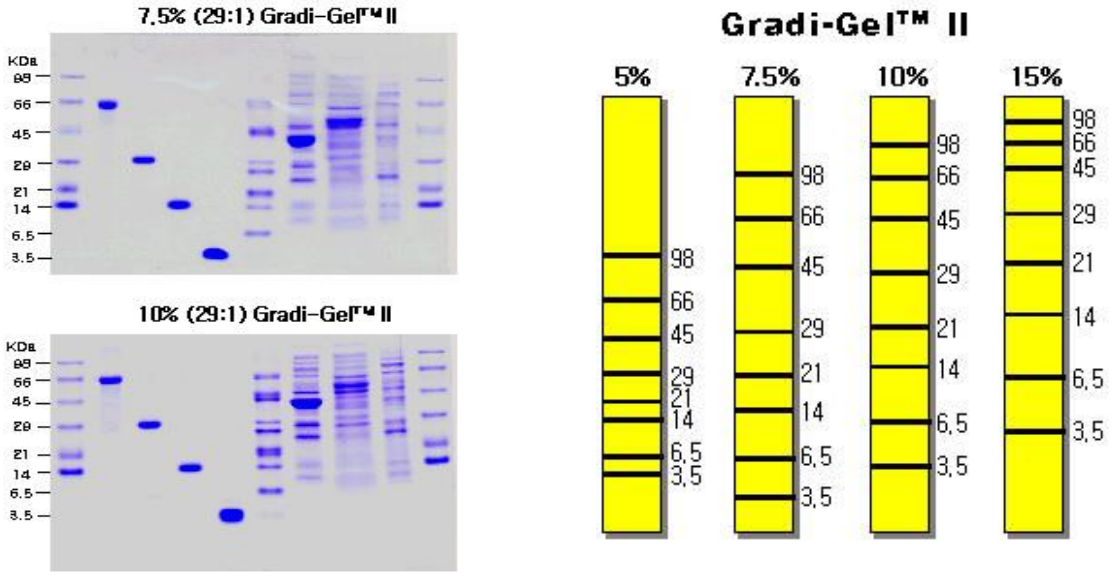

Migration pattern of Gradi-Gel™ II gradient PAGE analysis kit

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 10% running gel solution (29:1 acrylamide/bisacrylamide).

5 ml	Gradi-Gel™ II 2x running gel buffer
3.33 ml	30% Acrylamide/bisacrylamide gel stock (29:1)
5 μl	TEMED
0.1-0.2 ml	10% Ammonium persulfate
10 ml	**add DW to final vol. 10 ml**

   - Make sure for proper gel % before electrophoresis. See migration pattern of **Gradi-Gel™ II** gradient PAGE analysis kit.
   - SDS are not included in the kit, but it do not affect proper separation and high resolution of 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 10-30 min).
   - 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.

6. For the stacking gel solution (5%, 2 ml), mix the following:

1 ml	Gradi-Gel™ II 2x stacking gel buffer
0.333 ml	30% Acrylamide/bisacrylamide gel stock
2 μl	TEMED
0.02 ml	10% Ammonium persulfate
2 ml	**add DW to final vol. 2 ml**

7. 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.

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

9. 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.

10. Combine protein sample and 1× SDS gel-loading buffer (50 mM Tris-Cl, pH 6.8, 1% β- mercapto ethanol, 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.

11. 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 (8×10cm^2), electrophoresis has performed at constant voltage of **100-150 V**. It may consume **1.5-2 hrs** to complete the electrophoresis.
   - High voltage or current may result in generation of air bubbles in gels during electrophoresis affecting the resolution.