Fine-gel SDS-PAGE Gel Solution (15%)

F1503

Storage

Store at 4°C.

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- ***Fine-gel*** SDS-PAGE Gel Solution (15%)

Shipping Condition

Ship with ice pack.

Introduction

The ***Fine-gel*** SDS-PAGE Gel Solution (15%) is a TEMED free, ready-to-pour pre-mixed solution of acrylamide, bis-acrylamide, buffer, and SDS that enable ultra-fine resolution of protein bands by denaturing PAGE. This novel formulation requires only the addition of APS and allows separation of proteins from 2kDa to 100 kDa on the same mini-gel with less prep work than traditional SDS-PAGE. This product provides a superior resolution and a significant time savings, with no need to weight hazardous acrylamide powder or pour stacking gel sepsrately The gel is compartible with post-electrophoresis applications such as Western blotting, MALDI analysis, protein sequencing and other downstream applications. It is also suitable for staining with all commonly used dyes such as Coomassie Brilliant Blue, silver stain and fluorescent dyes.

Reagents

* **Provided by supplier**

Fine-gel SDS-PAGE Gel Solution (15%)

* **Provided by user**

Ammonium Persulfate (APS)
Sample Loading buffer
Gel Running Buffer

Precaution

This product contains acrylamide that is a potent, cumulative neurotoxin and absorbed through the skin. When working with this solution, always wear suit-able lab coat, disposable gloves, and protective goggles.

Protocol

The procedure is carried out at room temperature, unless stated otherwise.

1. Prepare gel solution

* Determine the volume of the gel mold (This information is usually provided by the manufacturer). Pour the appropriate volume of *Fine-gel* SDS-PAGE Gel Solution into a conical tube. Add 50-100 ul of 10% Ammonium Persulfate per 10ml *Fine-gel* SDS-PAGE Gel Solution.

Note: The required amount of 10% Ammonium Persulfate depends on temperature. If temperature is higher than 25°C, add 50 ul of 10% Ammonium Persulfate per 10ml of *Fine-gel* SDS-PAGE Gel Solution. If temperature is lower than 25°C, increase the amount of 10% Ammonium Persulfate by 2 times to save time during polymerization.

* Thightly cap the tube and gently invert the solution to mix (DO NOT VORTEX!). Pour the solution between the glass plates. The solution should be poured to the top of the plates. If stacking gel is not needed. To get a better resolution, we recommend using *Fine-gel* Stacking gel.

Note: *Fine-gel* SDS-PAGE Gel Solution with *Fine-gel* Running buffer is designed not to use stacking gel. But if necessary use *Fine-gel* Stacking gel.

* Immediately insert clean comb, being careful to avoid trapping air bubbles. Place the gel to polymerize completely at room-temperature, about 15 minutes to 1 hr.

* After polymerization is complete, remove the comb carefully and rinse wells with water or running buffer to remove unpolymerized acrylamide and residual gel pieces.

* Assemble gel system and add sufficient 1X *Fine-gel* Running buffer diluted from the supplied 10X stock to the top and bottom reservoirs. For the best resolution, use the *Fine-gel* Running buffer.

2. Sample preparation

* Dilute 1 part Laemmli sample buffer (4X) with 3 parts sample.
* Boil 3-5 minutes in water bath and cool on ice.
* Briefly Centrifuge the tube and load.

3. Electrophoresis

* Run gel at 100V for 60 minutes or until tracking dye reaches bottom of the resolving gel.
* Disassemble the apparatus and remove gel carefully from the plates.
* Proceed with protein detection or transfer.

Troubleshooting

Problem	Cause	Solution
	Air bubbles in the sample wells, or between gel and cassette, or at the bottom of the cassette.	Use a tansfer pipette to displace the air bubbles from the sample wells
	Sample contains appreciable carbohydrate	Remove the carbohydrate by enzymatic or chemical means
	Sample contains lipoproteins.	Use a gel with a large pore size at top of try adding a non-ionic detergent
Streaking	Poorly soluble or weakly charged particles (such as carbohydrates) in sample	Centrifuge sample
		Change pH of sample buffer
		Heat sample in the presence of SDS
Bands difficult to distinguish	Incorrect gel selection, sample overloading and insufficient cooling buffer	Select a gel that separates in the desired molecular weight range Reduce sample size Increase buffer volume in the outer tank For proteins of similar molecular weight, a 2-D seperation may be required.

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Troubleshooting

Problem	Cause	Solution
Sample spreading across gel	Excess salt in the sample	Reduces salt by dialysis or ultra-filteration
	Too much protein applied to the gel	Optimize the amount of protein applied to the gel
Protein denaturation and band inversion	Excess heating	Start with chilled buffer (<15°C)
Diffuse protein zones in the gel after staining	SDS present in the gel	Wash gel extensively (3x5 mins) with ultrapure water and use 30% methanol to destain gel
	Protein bands are diffusing	Use 10% TCA to fix the proteins in the gel
Buffer front and proteins migrate only partly down the gel and distored	Incorrect running buffer used (e.g., Tris-glycine-SDS)	Use only Tris-HEPES-SDS Running Buffer (100mM Tris, 100mM HEPES, 1% SDS, pH 8.0)
Run too slow	Buffers too concentrated	Check the buffer protocol: Dilute if necessary
	Current too low	Increase voltage by 25-50%
Gel does not polymerize	Temerature too low	Cast room temperature
	Ammonium persulfate is off	Use fresh ammonium persulfate
Gel brittle	Excess catalysis	Gel polymerizes in less than 15 minutes
		Reduce ammonium persulfate

Related Products

Product Name	Cat No
Albumin, Ultra pure Bovine Serum Albumin	A0100
Xpert Protease Inhibitor Cocktail Solution (100X)	P3100
Xpert 2 Prestained protein marker	P8502
Tween 20, Molecular Biology Grade	P9100
West-Ez Blocking Buffer, 3% BSA	W3710
West-Q Chemiluminescent Substrate Kit	W3650
West-Q Chemiluminescent Substrate kit, Plus	W3651
West-Ez Stripping Buffer	S2100

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