

# JULE SNAP-A-GELS™

## OPERATOR'S MANUAL PRECAST POLYACRYLAMIDE LINEAR AND GRADIENT MINI AND LARGE SIZE GELS

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11/18/02 EDITION

## 1. INTRODUCTION

Jule Snap-A-Gels are produced with the highest grade of materials and workmanship. Special equipment and techniques provide gradient gels with true linearity. Gradient gels are individually poured to eliminate the inherent errors of batch processing. Our methods assure high quality and reproducibility.

### CAUTION

Acrylamide monomer has been shown to be a neurotoxin in animals. Polymerized acrylamide, which is the form found in the gels, is not toxic. Although the gels are rinsed thoroughly, free monomer may still be present on the gel. Always wear safety glasses and protective latex gloves when handling electrophoresis gels.

Jule gels are designed to operate in equipment produced by other manufacturers. If in doubt concerning a particular procedure, follow the instructions provided with your apparatus. Jule gels are intended for research purposes only.

### STORAGE

For best results, store gels flat at 4° C. DO NOT FREEZE GELS. Gels are individually packaged with an expiration date printed on the label.

## 2. RUNNING THE GELS

### A. INTRODUCTION

Jule Precast Gels may be purchased with or without preformed sample wells. This provides researchers with a wide range of options. If gels were purchased without sample wells, refer to the stacking gel section of this manual.

### B. SAMPLE PREPARATION

Both concentration and the volume of sample loaded will affect performance. Sample overload results in over staining and hence, poor resolution. The amount of protein loaded depends upon the sensitivity of the detection method to be used. Coomassie stain, for example, detects protein from .1 to 1.0 micrograms ( $\mu\text{g}$ ) per band. Sample is generally made in a ratio of 1  $\mu\text{g}$  of protein per 1 microliter ( $\mu\text{l}$ ) of sample buffer. In contrast, silver stain detects down to 1.5 nanograms per band. In general, sample loading volume should be less than 1/2 the height of the sample well.

For best results refer to the recommended sample loading volumes given below. One should test with different loading concentrations and volumes in each well on a single gel to determine optimum loading for best resolution.

<b>GEL TYPE</b>	<b>RECOMMENDED SAMPLE* VOLUME LOADING (<math>\mu\text{l}/\text{well}</math>)</b>	<b>TOTAL WELL VOLUME (<math>\mu\text{l}/\text{well}</math>)</b>
<b>MINI GELS:</b>		
0.75mm 10 wells	2 to 15	25
15 wells	2 to 10	18
1.50mm 10 wells	3 to 30	50
15 wells	3 to 20	37
<b>LARGE GELS:</b>		
0.75mm 10 wells	10 to 140	150
15 wells	10 to 65	80
1.50mm 10 wells	20 to 300	320
15 wells	10 to 190	220

\*The recommended amount of sample per band is 0.10 to 1.0  $\mu\text{g}$  per band for Coomassie stained gels. For best results, load sample buffer into unused sample wells.

\*Tricine gels, which are typically used to resolve smaller proteins in the range below 50,000 Daltons, are loaded with higher protein concentrations. Approximately 2 to 5  $\mu\text{g}$  of protein per band is recommended due to partial loss during staining.

### C. SAMPLE BUFFER

Sample buffers are made of lower ionic strength than the running buffer so that a higher voltage is produced across the sample zone. The following table shows the recipes of 1X concentration sample buffer that can be mixed in a ratio of 1  $\mu\text{g}$  dry protein sample per 1  $\mu\text{l}$  of sample buffer, which is a useful concentration when staining with Coomassie Blue. The 2X sample buffer is prepared with 10% glycerol in order to increase the density of the sample. The more dense solution will allow the sample to sit at the bottom of the sample well and minimize sample buffer mixing with the running buffer during loading.

#### 1X SAMPLE BUFFER DESCRIPTION - DENATURING

0.062 M Tris-HCl, pH 6.8, 2% SDS\*

0.01% Bromophenol Blue

10% Glycerol

5% 2-mercaptoethanol *or* 0.1 M DTT (dithiothreitol)

**2X DENATURING SAMPLE BUFFER RECIPE (liquid samples):**

2.5 ml 0.5 M Tris, pH 6.8  
4.0 ml 10% SDS (w/v)  
2.0 ml Glycerol  
1.0 ml 2-mercaptoethanol or .155g DDT (dithiothreitol, FW - 154.2)  
2.0 mg Bromophenol Blue  
Add ultrapure water to a final volume of 10 ml. Divide into aliquots and freeze.

Samples are mixed 1:1 with 2X sample buffer (recipe shown above) in a small Eppendorf tube and heated to 100 degrees C for 2-3 minutes in a boiling water bath prior to loading onto a gel.

\*Higher concentrations of SDS (up to 5%) can be used for samples difficult to make soluble.

**2X NON-DENATURING (Tris-HCl) SAMPLE BUFFER RECIPE (liquid samples):**

Simply omit the SDS and 2-mercaptoethanol (or dithiothreitol, DDT) in the above recipe. SDS is also omitted from the running buffer when running non-denaturing gels.

**2X NON-DENATURING (Native, TBE) SAMPLE BUFFER:**

Dilute native (TBE) running buffer 5 times with ultrapure water. Add 20% Glycerol and .02 % dye.

Samples are mixed 1:1 with native sample buffer. DO NOT HEAT THE NON-DENATURED SAMPLE PRIOR TO LOADING.

**TRICINE GELS (DENATURING AND NON-DENATURING) SAMPLE BUFFER.**

See Tricine Gel Section.

**D. SAMPLE LOADING**

- 1). If gels were purchased with sample wells and a comb, gently remove the 10-well or 15-well comb from the top of the gel by sliding it with a slow steady motion straight up and out from between the cassette. It is best to do this under running water. Be careful not to distort or tear any of the wells when removing the comb from the gel.
- 2). Carefully flush out the wells with ultrapure water from a squirt water bottle to assure there is no residual acrylamide present.
- 3). Glass Cassettes Only: For proper sealing, be sure that the gel side spacers are flush with the top plate and sides of the gel before clamping the gel into its holding fixture. Gently adjust spacers, if necessary. Leave tape on sides of gel.
- 4) Using a permanent marker of any type, draw lines underneath each well on the outside of the tall plate so that it will be easier to see the well locations when loading sample.
- 5) Insert the Jule Precast Gel(s) into your electrophoresis apparatus according to the manufacturer's instructions. Remember to snap off bottom tab on Jule Snap-A-Gel Cassettes.

**ATTENTION BIO-RAD MINI PROTEAN II APPARATUS USERS:**

*When assembling the plastic cassette onto the gel clamping assembly, DO NOT OVERTIGHTEN THE SCREWS. Turn the 4 white screws until the push plate contacts the cassette and then rotate screw 1/8 turn (45 degrees) more. The screws should be just barely secure.*

*If you are having trouble sliding the cassette into the gel clamping assembly, you may need to back out the small center screw that is located between the bigger outside screws. This small screw sometimes gets in the way of the cassette.*

- 6) Fill the upper buffer tank with running buffer until the sample wells are filled. Check for leaks. If any leaking occurs, apply a small amount of grease such as Lubri-Seal (Thomas Scientific) or Cello-Seal (Fisher Scientific) to the seal of the silicon gasket. Add buffer to the lower tank. Remove any bubbles trapped under the gel sandwich by stirring lower buffer.

- 7) Using a microliter syringe, carefully load an appropriate amount of sample into the bottom of the sample well. **For best results, load 1X sample buffer in any wells that do not contain sample.** Turn power on immediately after loading. Refer to the recommended power settings for Jule Gels (See Section G). **Constant voltage is recommended for best results.**

## E. RUNNING BUFFER (TANK BUFFER)

**RUNNING BUFFER SHOULD BE MADE FRESH WEEKLY FOR BEST RESULTS.**

### **Tris-Glycine-SDS Buffer, Denaturing (D-gels)**

0.025 M Tris, 0.192 M Glycine,

0.1% SDS. Final pH will be 8.3 - 8.45 (**Do not adjust pH!**)

#### **Recipe: (4 Liters, 1X) DO NOT ADJUST pH!**

12.0 g Tris

57.6 g Glycine

4g SDS or 40 ml 10% (w/v) SDS Solution

Add ultrapure water to a final volume of 4.0 liters.

### **Tris-Glycine, Non-Denaturing Gels (W-gels), Do not adjust pH!**

Same as above except omit the SDS.

### **TBE (1X, Native, N gels)**

0.090 M Tris, 0.089 M Boric Acid, 0.0026 M EDTA

Recipe (5X TBE)

54.5 g Tris

4.9 g Na<sub>2</sub>EDTA-H<sub>2</sub>O OR 3.8 g EDTA

Bring up to 900 ml with ultrapure water. Add solid Boric Acid

until pH 8.3 (approximately 27.5 g). Then bring up to 1-Liter final volume.

## F. TRICINE GELS

Oligopeptides with molecular masses below about 10,000 are not well resolved by SDS-PAGE using the Laemmli buffer system, even with 15% or 20% polyacrylamide gels. Glycine is the tracking ion in the Laemmli buffer system. By replacing glycine with the tricine, small SDS-polypeptide complexes separate from SDS and so are resolved. Tricine migrates much faster than glycine in the stacking gel at the pH values, which occur during electrophoresis. As a consequence, the stacking limit is shifted to the low-molecular-mass range.

Brilliant Blue G is used as the tracking dye with tricine gels since it runs a little faster than the smallest proteins in all gels, whereas the commonly used bromophenol blue runs behind the smallest proteins in 10% acrylamide/bis-acrylamide gels, but before them in high-percentage acrylamide gels. If bromophenol blue is used in the sample buffer, the gel should be stopped before the dye reaches the bottom of the gel (1-inch). Jule tricine gels are ideal for the separation of low molecular weight proteins below 50 kilo-Daltons.

### **Tricine Gel Denaturing Running Buffer (1X)**

CATHODE BUFFER/UPPER BUFFER CHAMBER: (Do not adjust pH)

0.1 M Tris, 0.1 M Tricine, 0.1% SDS

Final pH should be 8.2 - 8.3

#### **Tricine Gel, Cathode (upper) Buffer Recipe:**

Trizma Base      12.11g

Tricine            17.92g

SDS                1.0 g

Dissolve and bring up to 1 Liter using ultrapure water. The solution should be approximately 8.2 pH.

**ANODE BUFFER, LOWER BUFFER CHAMBER:** 0.2 M Tris, pH 8.9

**Tricine Gel Anode (lower) Buffer Recipe:**

Trizma Base 24.22g

Dissolve to 850 ml with ultrapure water. Adjust to pH 8.9 with concentrated HCl. Bring to final volume of 1 Liter with ultrapure water.

**Tricine Gel Sample Buffer Recipe (2X):**

For non-denaturing gels do not add the SDS, DTT or 2-mercaptoethanol components.

8% SDS

24% Glycerol

0.1 M Tris, pH 6.8

4% 2-Mercaptoethanol or .31g DTT (FW 154.2)

.01% Brilliant Blue G dye

**Sample Loading:**

Incubate protein sample for 30 minutes at 40 degrees C in 1X sample buffer. In the case of small proteins (1-3k Daltons size range) an amount of 2-5 µg of protein per band should be applied because of partial loss during the staining and destaining procedure.

**G. POWER SETTINGS**

Follow the manufacturer's safety instructions for the electrophoresis chamber being used. JULE RECOMMENDS RUNNING GELS AT CONSTANT VOLTAGE USING THE SETTINGS BELOW. Increase the voltage until the STARTING CURRENT equals the values below. The current will decrease as the run progresses.

<u>GEL TYPE</u>		<u>VOLTAGE SETTING (ref. only)</u>	<u>STARTING CURRENT mAMPS PER GEL</u>	<u>RUN TIME HOURS</u>
<b><u>Tris Glycine Gels:</u></b>				
<b>Mini Gel:</b>	0.75mm	150-170	30	0.75 - 1.0
	1.50mm	150-170	35	0.75 - 1.0
<b>Large Gel</b>	0.75mm	100	18	4 - 6
	1.50mm	100	30	4 - 6
<b><u>Tricine Gels:</u></b>				
<b>Mini Gel:</b>	0.75mm	100	30-40	1-1.5
	1.50mm	100	55-60	1-1.5
<b>Large Gel:</b>	0.75mm		15-20	8-12
	1.50mm		30-40	8-12

Tracking dye will help monitor the end of the run. Running times may vary depending on gel concentration, apparatus being used, gel type, and gel thickness.

**3. STAINING GELS**

After completion of the run, disconnect the power supply and remove the cassette(s) from the running apparatus according to the manufacturer's instructions. Slowly pry the plates apart. You may now fix and stain (Coomassie Blue) gel(s) in stain solution for approximately 1 hour. This is followed by destaining the gel(s) for approximately 2-4 hours, until the desired contrast between protein bands and background is reached.

NOTE: For faster destaining include a small sponge or Kimwipes in the destain solution. When using this technique one does not have to change the destain buffer as often. Simply change the sponge or Kimwipes when they become saturated with stain.

**STAINING SOLUTION (500 ml)**

0.5 g Coomassie Blue  
250 ml Methanol  
50 ml Acetic Acid  
200 ml Ultrapure water

**DESTAINING SOLUTION (500 ml)**

150 ml Methanol  
300 ml Ultrapure water  
50 ml Acetic Acid

For a list of other staining or detection protocols consult *Gel Electrophoresis of Proteins* by Rickwood and Hames, available from Sigma Chemical Co.

## 4. BLOTTING

Many methods are available for the transfer of proteins from polyacrylamide gels to membranes ("blotting"). Specific details depend on the type of membrane used (e.g. , nitrocellulose or nylon), the type of gel used (denaturing or non-denaturing), and the type of transfer desired (capillary or electrophoretic). The electrophoretic transfer method is rapid and takes place with high efficiency. For this method, the elements of the apparatus are assembled in the following order:

1. an electrode (cathode)
2. a foam pad
3. sheet(s) of filter paper
4. the gel
5. the transfer membrane
6. sheet(s) of filter paper
7. a foam pad
8. another electrode (anode)

Gels purchased with SDS or SDS present in the sample buffer will produce a negative charge on the protein. Proteins will migrate toward the anode. Therefore the blotting paper is placed on the anode side of the gel.

Care must be taken to remove any air bubbles from between adjacent elements (especially between the gel and the membrane). This whole assembly is lowered into a transfer chamber containing transfer buffer (25 mM Tris, 192 mM glycine, 20% (v/v) methanol, pH 8.3). With SDS-denatured proteins, transfer proceeds toward the anode (the (+) terminal, by convention it is the red power supply terminal) thus, the membrane should be positioned between the gel and the anode.

Transfer is ordinarily carried out at an applied potential of 100 volts (with electrodes 11-12 cm apart) and with a resulting current of 0.6 - 1.2 amps (with chamber cross-section of 20 cm X 25 cm). The temperature of the chamber during transfer should not exceed 60 degrees C. The chamber should be controlled by a cooling system, if necessary. Complete transfer usually requires about 30 minutes. More detailed instructions are available from suppliers of membranes and transfer apparatus.

Detection of transferred components on blots can be performed with the usual protein stains (Amido Black, Coomassie Blue, Silver Stain), by autoradiography if the proteins are radiolabelled, and by immunological detection.

A standard reference on blotting is: Towbin, et al., *proc. Natl. Acad. Sci. U.S.A.* 76, 4350. (1979).

## 5. DRYING GELS

In order to preserve a protein gel, it is possible to dry the gel using either a commercial gel drier or one made in the laboratory. If the polyacrylamide gels are dried down without any support they will shrivel. However, by drying down into a filter paper backing, the gel dimensions are preserved as the gel attaches itself to the paper support. For rapid drying, the temperature control of most dryers is pre-set at 80°C. Best results are obtained if the gel is soaked overnight in a solution containing 5% glycerol (v/v) and 10% ethanol or methanol (v/v). This prevents gel cracking.

Concentration gradient gels (e.g., 4-20% acrylamide) can be dried successfully if they are first soaked in 20% methanol (v/v) and 10% glycerol (v/v) from at least 1 hour to overnight. **THE DRYING TEMPERATURE SHOULD BE REDUCED SO THAT DRYING OCCURS MORE GENTLY OVER A LONGER TIME PERIOD.** This helps reduce cracking. The Bio-Rad Gel Dryer works very well on the bottom heating option (slow heat increase and then quick decrease). Amersham's (Hoefer) air gel-drying system, called the Easy Breeze™ is also recommended to reduce gel cracking and wrinkling.

## 6. POURING STACKING GELS

If you have ordered a gel without preformed sample wells, you will need to pour a stacking gel on top of the resolving gel.

Mix the appropriate stacking solution (see stacking gel recipe below) and degas for approximately 15-20 minutes. After adding appropriate amounts of ammonium persulfate & TEMED, the solution will polymerize in approximately 10-30 minutes. Using 2/3 of the recommended level of TEMED and APS can decrease the rate of polymerization.

Open the gel foil pack using scissors and pour off any buffer from the top of the precast gel. Using a pipette rinse the top of the resolving gel with ultrapure water and then with stacking gel solution containing 10% APS and TEMED to improve adherence between the stacking gel and resolving gel. Carefully fill the cassette with the stacking solution and insert the comb, avoiding trapped air under the teeth of the comb. Add gel solution until solution reaches the top of the cassette.

Allow the stacking gel to polymerize for a minimum of 30 minutes prior to removing the comb. Cold temperature decreases the rate of polymerization. If the gel was stored in the refrigerator, allow extra time for polymerization of the stacking gel.

Gently remove comb (preferably under running water) and rinse sample wells with ultrapure water. The gel may now run in your electrophoresis apparatus. Refer to the section on sample loading.

## 7. STACKING GEL RECIPES

### 4% STACKING GEL RECIPE FOR TRIS-GLYCINE DENATURING GELS

2.0 ml Acrylamide stock(40%)  
2.0 ml 10 X Tris-HCl buffer (pH 6.8)  
15.7 ml Ultrapure water  
0.2 ml 10% SDS (w/v)  
20.0 ml Total

DEGAS FOR 15 MINUTES AND ADD 150 µL 10% APS & 15 µL TEMED

### 4% NON-DENATURING GELS

For Tris-HCl buffer based Non-Denaturing (W) gels omit the SDS from the recipe above.

For TBE buffer based Non-Denaturing (N) gels substitute 4.0 ml of 5X TBE buffer, omit SDS, and add 13.7ml of ultrapure water to bring to final volume of 20ml in the above recipe.

### 4% STACKING RECIPE FOR DENATURING TRIS-TRICINE GELS

2.0 ml Acrylamide stock (40%) (See above)  
6.7 ml 3X Tris Buffer (See below)  
0.2 ml 10% SDS  
11.1 ml Ultrapure water  
20.0 ml Total

**3X Tris Buffer Stock:**

Dissolve 36.35 g Tris to 75 ml with ultrapure water, pH to 8.45 with concentrated HCl and bring up to 100 ml final volume.

**STOCK REAGENTS FOR STACKING GELS**

40% Acrylamide stock:

39 g Acrylamide

1 g *bis*-acrylamide

Dissolve to 100 ml with ultrapure water and filter. Store at 4°C in a brown bottle. Discard after 1 month.

**10X Tris Buffer:**

Dissolve 34.1g of Tris to 180 ml with ultrapure water, titrate to pH 6.8 using approximately 24 ml of concentrated HCl acid. Bring solution to 225 ml with ultrapure water and filter. Store at room temperature.

**10X TBE Buffer:** pH 8.3

27.0 g Tris

13.8 g Boric Acid

2.3 g EDTA

Dissolve to 250 ml with ultrapure water and filter. Store at room temperature.

**10% Ammonium Persulfate (APS):**

Dissolve 0.1 g Ammonium Persulfate in 1.0 ml ultrapure water. Keep at 4°C.

*Prepare fresh, discard after 24 hours*

## 8. MOLECULAR MASS DETERMINATION

**SDS-Denatured Proteins:**

Molecular mass estimations can be made through the use of known molecular mass standards, available in several ranges from commercial sources. At the completion of destaining a reference graph can be made by plotting the relative mobility,  $R_f$  (the distance traveled by the protein of interest through the gel in relation to a reference point such as the tracking dye) of the standards versus the Log base 10 of their known molecular mass. The molecular mass of the unknowns can be estimated from a graph by plotting the  $R_f$  of the unknown onto the same graph.

With uniform concentration polyacrylamide gels, there is a linear relationship between log base 10 molecular mass and  $R_f$  value or distance migrated by the SDS-polypeptide complex. However, with linear concentration gradient gels, the linear relationship is between log base 10 molecular mass and log base 10 polyacrylamide concentration (%T). After staining, the position of the protein bands is measured and %T calculated for each. For example, a polypeptide, which has migrated halfway through 5-20% polyacrylamide gel, will have reached a %T for the standard protein then yields a standard curve from which the molecular mass of the sample proteins can be determined.

Rothe and Purkhanbaba, *Electrophoresis*, 3. 43 (1982) have also shown that the molecular masses of proteins separated in linear SDS polyacrylamide gradient gels can be estimated from plots of log base 10 molecular mass (M) versus the square root of the migration distance. This relationship is time independent so that electrophoresis can be stopped at the most appropriate time to gain optimum resolution of the protein bands of interest.

**Native Proteins:**

Native proteins migrate in uniform concentration gels and in gradient gels, according to both their size and charge. The molecular mass of native proteins electrophoresed in a linear polyacrylamide gradient gel is best estimated by measuring the rate of its migration through the gel. Refer to the article by Roth and Maurer (1986) in *Gel Electrophoresis of Proteins* (ed. M. T. Dunn) who have described the determination of the molecular mass of native proteins in gradient gels at some length.

## 9. TROUBLE SHOOTING GUIDE

PROBLEM	POSSIBLE CAUSE	POSSIBLE SOLUTION
1) <b>Gels smile</b> <b>Side lanes curl up</b>	Power too high	Decrease power settings See Power Settings section
2) <b>Bands streaking</b> <b>vertically</b>	Sample overload or sample precipitated	Dilute sample. Decrease sample vol. loaded. Decrease acrylamide concentration. Decrease power. See (9) below. Make fresh running buffers.
3) <b>Band spreading</b>	Diffusion from wells in stack	Reduce load time. Increase power.
<b>bands spreading</b> <b>(widening) near</b> <b>bottom of gel</b>	Depleting electrolytes in lower buffer	Increase volume of lower buffer.  Load any empty sample wells with sample buffer.
4) <b>Skewed bands,</b> <b>distorted bands</b>	Salt in sample	Remove salt by dialysis, etc.
	Uneven gel heating.	Use cooled apparatus or reduce the current.
	Uneven gel interface.	Increase polymerization rate, overlay stacking gel carefully.
5) <b>Run time is too long.</b>	Current too low.	Increase voltage. Check power settings.
	Upper buffer level is below front plate.	Add upper buffer, upper buffer leak.
	Buffer too concentrated.	Check recipes and buffer concentration.
6) <b>Run time too short</b> <b>with poor resolution.</b>	Voltage too high Buffer too dilute.	Decrease voltage. Check power settings and buffer recipes.
7) <b>Two bands (doublet)</b> <b>where single band</b> <b>expected (SDS)</b>	Protein not fully reduced.	Prepare fresh sample buffer and/or increase reducing agent.

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8) <b>One band at dye front.</b>	Protein migrated together with dye front.	Increase gel concentration.
9) <b>Insoluble material in sample.</b>		
	<b>Non-denaturing buffers</b> Denatured protein.	Remove by Centrifugation.
	<b>SDS containing buffers.</b> Too little SDS, and/or reducing agent, pH too low.	Add more SDS or reducing agent (2-mercaptoethanol), increase pH, or add urea.
10) <b>Failure of sample to form a layer at bottom of sample well.</b>	Glycerol or sucrose was omitted from sample buffer. Residual acrylamide in well.	Add more glycerol Remove excess gel from wells.
11) <b>Uneven or poor staining.</b>	Too little stain added, gel not in stain long enough.	Increase volume of stain Increase staining time Make new stain solution.
12) <b>Stained bands lost after destaining.</b>	Gel left too long in destain. Improper fixative/ recipe.	Reduce destain time. Use a better fixative.
13) <b>Metallic sheen or slight film on gel.</b>	Solvent evaporated causing dye to dry onto gel.	Quick rinse in 50% methanol or gently swab gel surface with methanol soaked tissue paper.
14) <b>Blue blotches near gel borders.</b>	Fingerprints caused by gel handling.	Always wear gloves.
15) <b>Same protein bands across all gel tracks.</b>	Contamination of the sample buffer.	Make new sample buffer.
	Cassette is clamped too tightly (Bio-Rad Apparatus), causing bowing of cassette.	Tighten 1/8 of turn.
16) <b>Protein bands observed in adjacent lanes.</b>	Sample overflow.	Reduce the volume of sample loaded.
17) <b>High background of protein staining with indistinct protein bands.</b>	Extensive sample proteolysis in non-denaturing gels	Work at low temperatures and/or use protease inhibitors. With SDS PAGE, check that sample is being heated to 100°C for 2 minutes.
18) <b>Sample floats out of sample well.</b>	Running buffer is too concentrated or glycerol was omitted.	Check concentration of running buffer. Typically 1X conc. for Tris-glycine gels. Add glycerol to sample buffer.

<b>19) Heavily stained band at gel origin:</b>		
<b>denaturing gel</b>	Aggregated protein in the sample prior to electrophoresis	See (9) above. Use a lower acrylamide concentration.
<b>non-denaturing gel</b>	Precipitation of protein due to formation of highly concentrated zones in stacking gel during electrophoresis	Use less concentrated samples with a continuous buffer system.
<b>20) Irreproducibility of the protein band pattern</b>	Sample preparation, proteolysis	See (17) above.
<b>21) Run taking unusually long time</b>	Buffers too concentrated, low current	Check buffer protocol, dilute if necessary. Check power settings.
<b>22) Gel slips out of cassette during run</b>	Cassette is clamped too tightly on Bio-Rad apparatus causing cassette to bow.	Tighten the 4 screws only 1/8 turn (45 degrees).
<b>22) Bands close in size do not separate well, or there is an overlap.</b>	Bands are running together.	Choose higher concentration of acrylamide. Use shallow gradient gel or single conc. gel. Increase sample conc. and load less volume of sample so there is a thin line entering gel. Use longer path length gel.

## 10. WARRANTY

JULE PRECAST GELS are warranted to be free from defects in materials and workmanship for a period prior to the expiration date printed on the packaging. Jule, Inc. will repair or replace and return free of charge any part thereof which is returned to its factory within said period, transportation prepaid by user, and which is found, upon inspection, to have been defective in materials or workmanship. This warranty does not include normal wear from use, it does apply to any product or part thereof which has been altered by anyone other than an employee of Jule, Inc., nor to any product thereof which has been damaged through accident, negligence, failure to follow operating instructions, use beyond the specified capacity of the product, misuse, or abuse.

Jule, Inc. reserves the right to change, alter, modify or improve any of its products without obligation whatever to make corresponding changes to any product previously sold or shipped.

The foregoing obligations are in lieu of all other obligations and liabilities including negligence and all warranties, or merchantability or otherwise, expressed or implied in fact or by law, and state our entire and exclusive liability and buyer's exclusive remedy for any claim of damages in connection with the sale of furnishing of goods or parts, their design, suitability for use, installation or operation. Jule, Inc. will in no event be liable for any special or consequential damages whatsoever, and their liability under no circumstances will exceed the contract price for the goods for which liability is claimed.

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