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High Speed Protein Separation by SDS-PAGE

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Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) is a widely used technique for assessing in a reproducible manner the relative mass of denatured polypeptide chains in a complex protein lysate as well as the purity of a protein preparation. Polypeptides differing in weight by a few hundred daltons can be separated by SDS-PAGE.

For more than 40 years, the Laemmli SDS discontinuous buffer system has been used with Tris-glycine polyacrylamide gels for high-resolution fractionation of protein mixtures.[1] The gel matrix acts as a molecular sieve, and the Tris-glycine-SDS running buffer provides the ionic strength for the electric field. When current is applied, proteins migrate in the electric field based on their net charge, size and shape of the protein or its subunits. If the voltage is set too high, the running buffer will become hot prompting the gel matrix to heat up and then break down causing protein bands to diffuse and lose sharpness. Standard Tris-glycine mini-gels prepared with the Laemmli recipe are generally run at 125 V using TG-SDS buffer[2] resulting in long electrophoresis run-times (60-90 min). Since these gels cannot be run at significantly higher voltage without generating excess heat, this fact provided an opportunity to develop a new buffering system that would permit protein electrophoresis at higher voltages without compromising the gel matrix.

We report a new electrolytic buffer solution which can be run at increased voltages without generating high heat and impacting the clarity of protein bands. Preliminary data shows protein electrophoresis run times <25 minutes for traditional 10% Tris-glycine mini-gels (precast and homemade) using our new proprietary FAST*Run*™ Tris SDS PAGE running buffer. Moreover, protein bands separated using FAST*Run* buffer are sharper, and a uniform concentration gel is now able to fractionate proteins over a range of molecular masses similar to a gradient gel. Finally, increasing the speed of protein electrophoresis enabled by our FAST*Run* buffer improves the overall throughput of the protein analysis workflow so that downstream applications such as Western blotting and protein identification can be accomplished in the same day.

FAST*Run*™ Tris SDS PAGE Running Buffer, 10X solution is available in 500mL or 1L size. For more information please visit: www.fishersci.com/FASTRunSDSBuffer

Gel Specifications

- Gel type: precast Tris-glycine gel[3] or homemade TG mini-gel[4]
- Acrylamide percentage: 10% and 16% gels or 4-20% gradient gel
- Gel dimensions: 10 cm x 10 cm
- Comb: 10 well
- Thickness: 1.0 mm or 0.80 mm

Running Buffer

- Tris-glycine-SDS (TGS) buffer, 10X solution[4]
- BP881 FAST*Run*™ Tris SDS PAGE Running Buffer, 10X solution (Fisher Scientific)

Apparatus

- XCell SureLock™ Mini-Cell Electrophoresis System (Thermo Fisher Scientific)
- Mini-PROTEAN® Tetra Cell or Mini-PROTEAN 3 Cell (Bio-Rad)
- FisherBiotech™ Vertical Electrophoresis System (Fisher Scientific)

Protein MW Standards, Cell Lysate and Stain

- BP3602 EZ-Run™ *Rec* Protein Ladder (Fisher Scientific), 10 – 200 kDa (14 protein bands)
- BP3603 EZ-Run™ Prestained *Rec* Protein Ladder (Fisher Scientific), 10 – 170 kDa (10 protein bands)
- LC5800 Novex® Pre-Stained Protein Standard (Life Technologies), 3.5 – 260 kDa (12 protein bands)
- LC5677 Mark12™ Unstained Standard (Life Technologies), 2.5 – 200 kDa (12 protein bands)
- PI26628 Low Range Protein Ladder (Thermo Scientific), 1.7 – 40 kDa (6 protein bands)
- *E. coli* cell lysate from BP4000 TransMax™ Competent Cells, FB5α (Fisher Scientific)
- BP3620 EZ-Run™ Protein Gel Staining Solution (Fisher Scientific)

In these experiments, we utilized electrophoresis tanks from two suppliers which were compatible with precast and homemade mini-gels. Speed of protein separation under different applied voltages was analyzed using 10% TG polyacrylamide gels with either traditional TGS running buffer or the new FAST*Run* Tris SDS PAGE running buffer.

Experiment 1: Speed of Separation

Protein gel electrophoresis was performed in 10% TG precast and homemade gels using 1X TG-SDS running buffer and 1X FAST*Run* buffer (**Fig. 1**). Partial separation of the protein ladder was observed after 25 min run time using the gel manufacturer's recommended 125V for TG-SDS buffer (**Fig. 1a**). However, complete separation of 10 protein bands (10 – 170 kDa) after 25 min at 200V occurred with the use of FAST*Run* buffer in precast and homemade gels (**Fig. 1b,c**). In contrast, about 90 min were required to separate the protein ladder in the 10% gel using TG-SDS running buffer (data not shown). A low MW protein ladder (1.7 – 40 kDa) was separated in 35 min on a 16% TG gel using FAST*Run* buffer (**Fig. 1d**).

Table 1. Gel Type and Electrophoresis Run Conditions in Experiment 1

Gel Type	Running Buffer	Voltage	Run time	Temperature (Cathode Chamber)
Fig. 1a – 10% TG Precast	1X TG-SDS	125 V	25 min	24° C
Fig. 1b – 10% TG Precast	1X FAST*Run*	200 V	25 min	34° C
Fig. 1c – 10% TG Homemade	1X FAST*Run*	200 V	30 min	31° C
Fig. 1d – 16% TG Precast	1X FAST*Run*	200 V	35 min	33° C

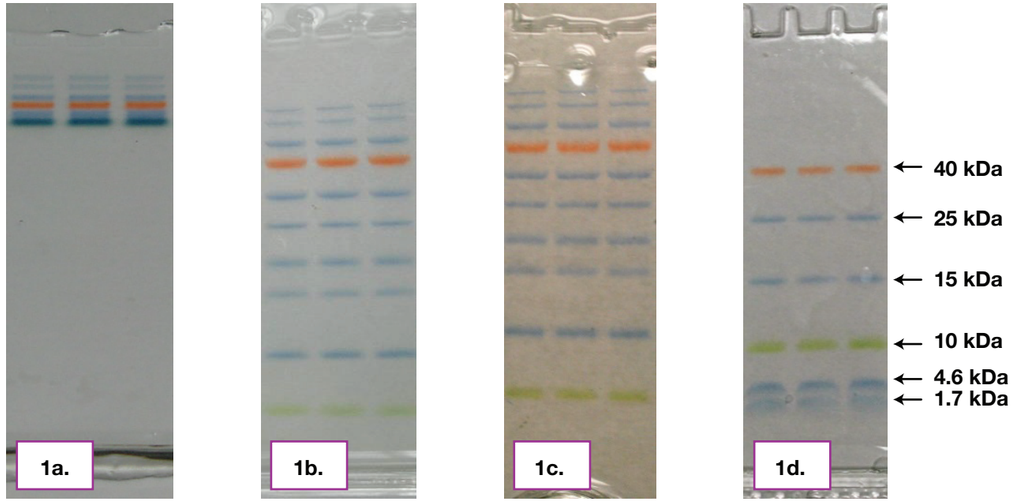


Fig. 1a-d. Electrophoretic separation of a protein ladder (BP3603) in a 10% TG precast gel after 25 min using TG-SDS running buffer (**1a**) and FAST*Run* buffer (**1b**). Same protein ladder separated in a 10% TG homemade gel after 30 min using FAST*Run* buffer (**1c**). Low MW range protein ladder (PI26628) electrophoresed on a 16% TG precast gel using FAST*Run* buffer (**1d**).

Experiment 2: Band Clarity at Different Voltages

FAST*Run* buffer is an electrolytic solution which can run at higher voltages without generating high heat and impacting the clarity of protein bands. For example, protein electrophoresis in 10% precast TG mini-gels (10 cm x 10 cm) was evaluated with increasing voltages (**Table 2**) using a full (700 mL) buffer tank of FAST*Run* buffer. Electrophoresis run times decreased from 23 min at 200 V to 13 min at 300 V without a significant increase to the operating temperature (buffer temperature in cathode chamber was about 35° C at end of run). Protein markers remained sharp with little evidence
of band diffusion (**Fig. 2a-c**).

Table 2. Electrophoresis Run Conditions in Experiment 2

Voltage	Run Time	Current (mA)		Temperature (Cathode Chamber)
		Start	End	
Fig. 2a – 200 V	23 min	98	54	34° C
Fig. 2b – 250 V	18 min	135	75	34° C
Fig. 2c – 300 V	13 min	169	109	36° C

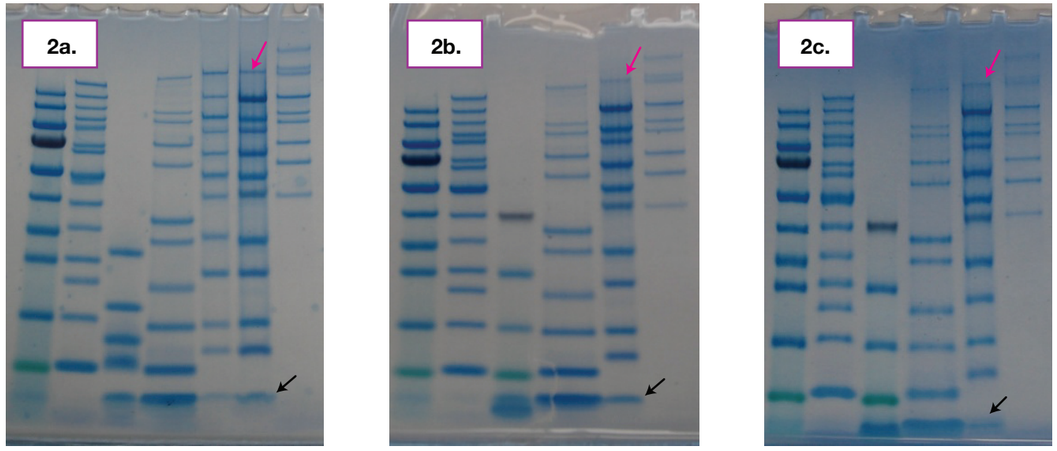


Fig. 2a-c. Decreasing electrophoresis run time with increased voltage in a 10% precast TG gel using FAST*Run* buffer: 23 min at 200 V (**2a**), 18 min at 250 V (**2b**), and 13 min at 300 V (**2c**). Protein bands are sharp and fully separated from 3.5 kDa (black arrow) to 260 kDa (red arrow).

Experiment 3: Separation Range

In the traditional Laemmli SDS-PAGE system, lower molecular weight (MW) proteins do not separate, i.e. they co-migrate within the electrophoretic migration front. This effect was readily observed in lane 5 of a 10% precast TG gel which was run with TG-SDS buffer (**Fig. 3a**). The 3.5, 10, and 15 kDa proteins co-migrated and were not distinguishable. However, the same gel run with FAST*Run* buffer showed complete separation of these lower MW proteins (**Fig. 3b**). Moreover, the separation range in this 10% TG gel using FAST*Run* buffer was equal to typical runs using 4–20% gradient gels.

Table 3. Electrophoresis Run Conditions in Experiment 3

Running Buffer	Voltage	Run time	Temperature (Cathode Chamber)
Fig. 3a – 1X TG-SDS	125 V	90 min	24° C
Fig. 3b – 1X FAST*Run*	200 V	25 min	32° C

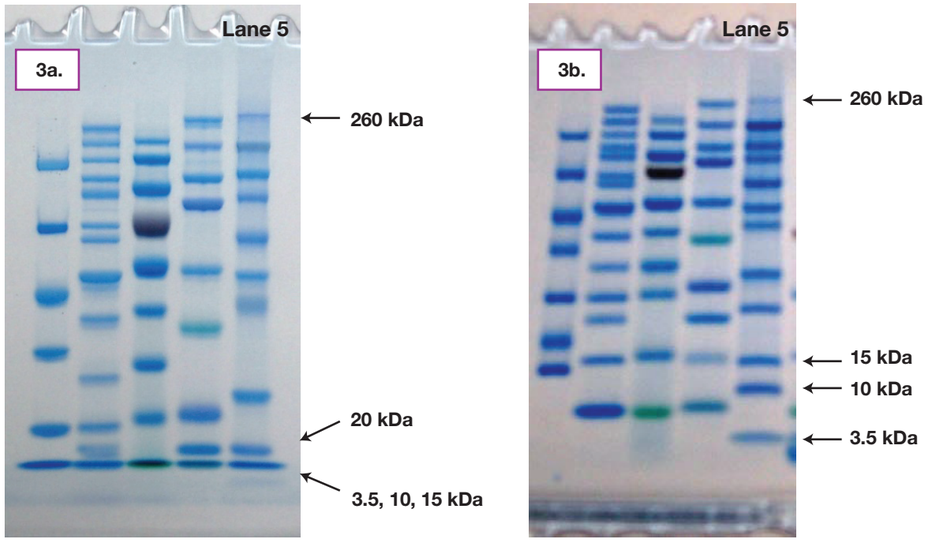
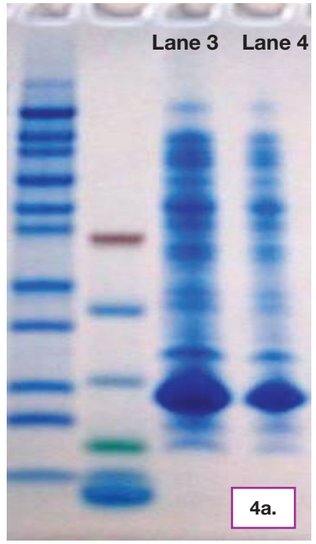


Fig. 3a,b. Poor separation of low molecular weight proteins (3.5, 10, and 15 kDa) occurred in a 10% precast TG gel using TG-SDS buffer (**3a**). However, FAST*Run* Tris SDS PAGE running buffer used with the same uniform 10% gel fractionated proteins over a wider range of molecular masses similar to a gradient gel (**3b**).

Experiment 4: Cell Lysate and Protein Separation

E. coli cell lysate was loaded on a 10% TG gel and electrophoresed using 1X FAST*Run* buffer (**Fig. 4a**). Lanes 3 and 4 had 10 µl and 5 µl, respectively, of *E. coli* lysate. The complex *E. coli* protein mixture fractionated well as did individual proteins such as carbonic anhydrase (29 kDa) and albumin from chicken egg white (45 kDa) shown in **Fig. 4b.**



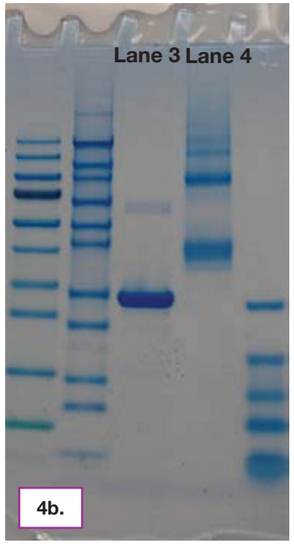


Fig. 4a,b. Separation of a complex protein mixture from *E. coli* lysate in lanes 3 and 4 (**Fig. 4a**) using 10% TG gel with 1X FAST*Run* buffer. Individual proteins isolated with FAST*Run* buffer protocol (**Fig. 4b**), carbonic anhydrase (29 kDa, lane 3) and albumin from chicken egg white (45 kDa, lane 4).

Experiment 5: Compatibility of FAST*Run* Tris SDS PAGE Buffer With Various Gel Tanks

FAST*Run* buffer was tested with different gel tanks and gel cassettes at 200V without generating excessive heat or compromising protein resolution. If multiple gels are run together, voltage and run time adjustments may be necessary – check the manufacturer's recommendation for maximum allowable voltage.

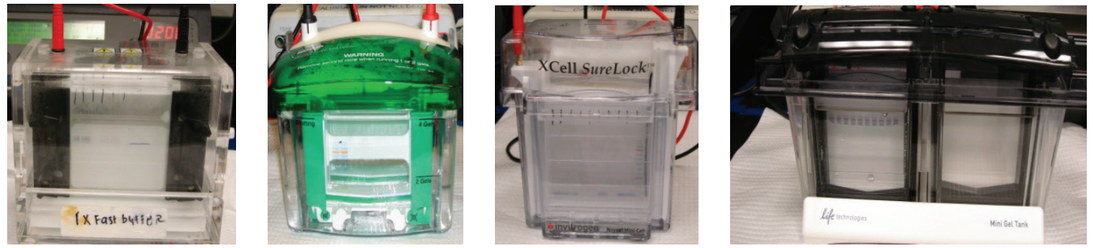


Fig. 5. Various manufacturers' gel boxes tested successfully with FAST*Run* Tris SDS Page running buffer.

Since the mid-1970s Laemmli's method based on a Tris-glycine-SDS buffer system has been widely used for analyzing protein mixtures by PAGE. Laemmli's gel and buffer system provides clear and accurate molecular weight estimation of proteins in complex samples from a wide variety of sources, but at the cost of long electrophoresis time. Recent improvements of the SDS-PAGE method to reduce total run-time have been limited primarily to high-priced precast gels. Indeed, the general industry point of view is that modification of electrokinetic methods involving only the polyacrylamide gel matrix may lead to advances in separation speed and resolution of proteins.

In this work, we demonstrate an improved Laemmli method which provides significant cut in electrophoresis run-time through patent pending modifications made solely to the running buffer system. Traditional TG polyacrylamide gels (precast or homemade) can be operated at higher voltages using the new FAST*Run* Tris SDS PAGE running buffer without generating high heat which may reduce the clarity of separated protein bands (**Fig. 1, 2**). We show that typical mini-gels (10 cm x 10 cm) cast with the Laemmli Tris-glycine recipe and run with the new buffer decreases electrophoresis run-time by 50 to 60% and improves band sharpening. In the protein characterization workflow, the speed to complete separation and identification of a protein is important. For some labs, it is not affordable to switch to specialized precast gels and buffering systems to improve lab throughput. Instead, a simple conversion to the unique FAST*Run* Tris SDS PAGE buffer for use with standard Tris-glycine gels provides the speed and resolution that researchers require at a reasonable cost.

A major drawback of the Laemmli SDS-discontinuous buffer system is that proteins smaller than 15 kDa are very poorly separated, i.e. they co-migrate within the electrophoretic migration front (**Fig. 3a**). However, a 10% TG gel run with FAST*Run* buffer permitted complete separation of the lower molecular weight proteins (**Fig. 3b**) which is similar to the resolving power of a 4-20% gradient gel[2] or a 10% gel with Tricine SDS running buffer.[5] Finally, the necessity of gradient gels for analyzing complex protein mixtures is less important since a uniform concentration gel can fractionate proteins using FAST*Run* buffer over a wide range of molecular masses in half the time (**Fig. 3b**).

References

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