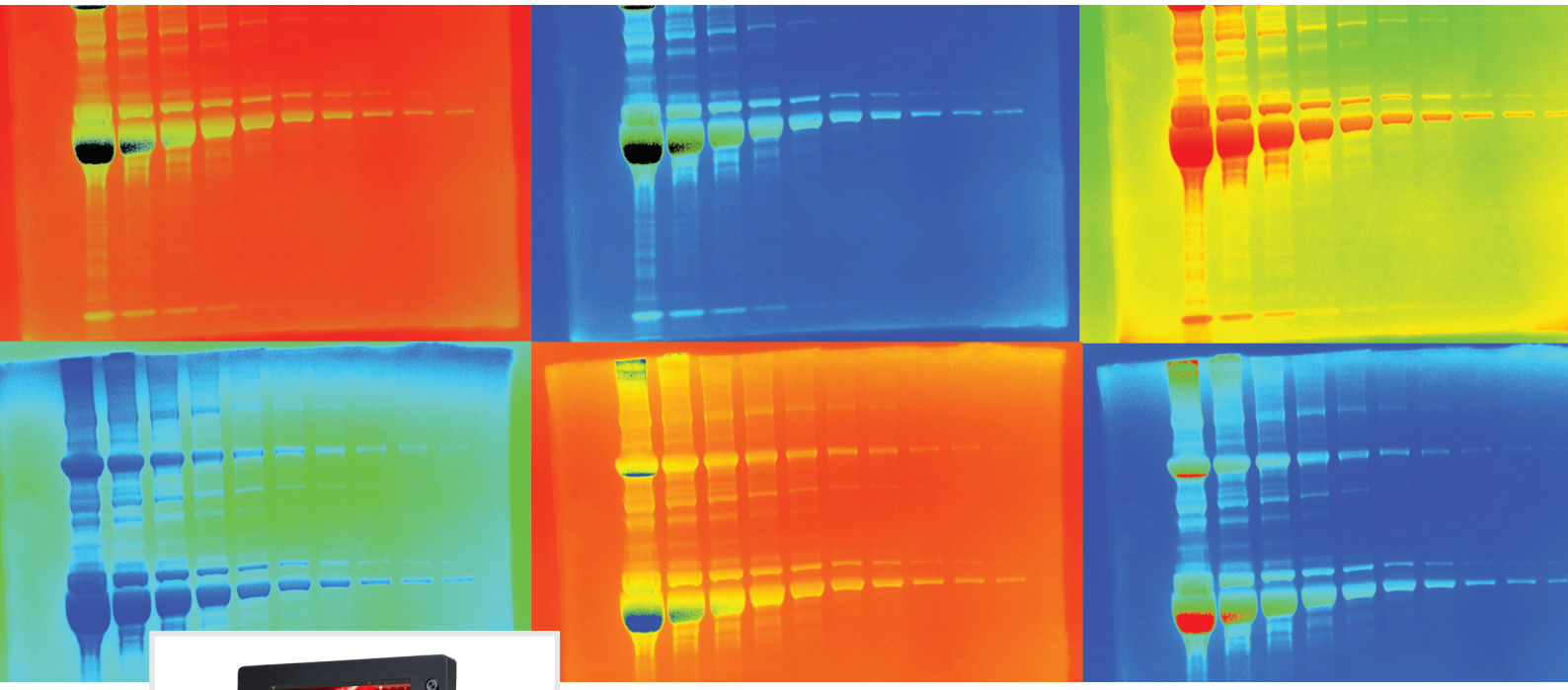

Task

Simplified high resolution imaging of protein samples

Solution

Analytik Jena's UVP GelSolo enables easy yet exceptional gel documentation with an intuitive user interface and a high resolution 5MP camera

Theory and Practice: Performing 1D SDS-PAGE

Introduction

Researchers have studied proteins since the 18th century[1], but several 1960's discoveries which demonstrated sodium dodecyl sulfate (SDS) could facilitate the separation of proteins, ushered in a new era of protein science. Researchers could now study complex mixtures of proteins or combine SDS with reducing reagents to separate purified multimeric complexes[2]. The introduction of the polyacrylamide slab gel in the late 1960s and early 1970s enabled simultaneous comparison of multiple samples[3,4]. Stack on an improved solubilization buffer[5], we arrive at present day SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). While there are many elaborations of traditional 1D SDS-PAGE, a common requirement is reliable documentation for analysis which requires high-quality precision instrumentation—quality and precision are mainstays of Analytik Jena's 50 year imaging history.

The UVP GelSolo is an entry-level imaging system well-suited for any laboratory, and provides exceptional documentation of nucleic acid and protein gels and beyond (contact our Applications Scientists for additional applications). Equipped with a 5 megapixel CMOS camera, the UVP GelSolo can capture the finest details in your samples. Below, we provide a brief overview of 1D SDS-PAGE, along with some expert tips to get the most out of your 1D SDS-PAGE gels.

analytikjena
An Endress+Hauser Company

Theory and Practice: Performing 1D SDS-PAGE

Electric Field

Unlike DNA molecules, proteins do not have uniform charges across the polypeptide. Instead, proteins are composed of non-polar, weakly polar, polar, and charged side chains or R-groups. This panoply of electrical potential causes proteins to migrate unpredictably under native conditions and therefore mass cannot be accurately determined unless combined with isoelectric focusing as in 2D gel electrophoresis—but this method is laborious, challenging, and unnecessary for most applications. Fortunately, by incorporating SDS, proteins are denatured and a uniform negative charge is imposed on the polypeptide, masking the intrinsic charges of the R-groups. SDS binds near uniformly (1.4 g SDS/1 g protein), so protein charge will approximate protein size. As in DNA gel electrophoresis, proteins are loaded onto a gel matrix and an electrical field is applied—negatively charged polypeptides will migrate toward a positive electrode according to their molecular weight (see Table 1). Depending on the type of gel used during SDS-PAGE, voltages will change. If a stacking and resolving layer are used, users generally run protein samples for approximately 30 minutes at a low voltage to improve stacking and, therefore, band resolution. The voltage is then increased and run for up to 2 hours. Alternatively, manufactured precast gels enable users to run gels at higher constant voltages with run times as short as 30 minutes.

Table 1: Resolving layer acrylamide percentage and recommended protein size ranges.

Concentration of acrylamide (%)	Protein size (kDa)
5	36-200
7.5	24-200
10	14-200
15	14-60

Gel Matrix and Gel Casting

Here we describe traditional homemade gels. As the name suggests, protein gels are composed of polyacrylamide. In the presence of cross-linking reagents and buffer, bis-acrylamide forms a polymer and quickly solidifies at room temperature. By changing the concentration of acrylamide, the sieving properties of the gel can be augmented as in DNA gel electrophoresis. Typical gels contain a stacking and resolving layer. The resolving layer is the region of the gel where the bands separate from one another. This region contains a higher concentration of acrylamide to retard the migration of the polypeptides. The stacking layer, on the other hand, has a lower concentration and promotes the stacking of the protein sample at the interface between the stacking and resolving layers. The resolving gel is poured first in between two slabs of glass in a gel casting tray. The resolving layer is overlaid with 2-propanol or 100 % ethanol to promote a flat stacking/resolving interface. After the resolving layer has polymerized, the alcohol is washed out repeatedly with water and the stacking gel is poured on top of the resolving gel. A comb is added as in DNA gel electrophoresis. Once the stacking layer has polymerized, the gel is ready for the addition of protein samples. Alternatively, users can purchase precast gels, which ensure reproducibility of experimental results.

Electrophoresis Buffers

There are several buffers for SDS-PAGE. As with DNA gel electrophoresis, buffers are required to conduct current during electrophoresis. Unlike DNA gel electrophoresis, discontinuous buffer systems are more common in SDS-PAGE. For example, a gel may contain Tris-HCl pH 6.8 in the stacking layer, Tris-HCl pH 8.8 in the resolving layer, and Tris-Glycine buffer pH 8.3 in the tank. In this system, glycine can take on two charge states. During electrophoresis, positively charged glycine in the tank buffer (pH 8.3) enters the stacking gel (pH 6.8) and bears a neutral charge—so it moves slowly in the polar field created by the electrodes. Chlorine in the gel buffer, in contrast, migrates much faster and creates a negative charge front ahead of the glycine. These opposing fronts bookend the protein samples, pinching them into a thin line as they migrate toward the stacking/resolving layer interface. At the interface, the charge state of glycine switches (pH 8.3) back to negative and its role is completed. The proteins enter the resolving layer as a tight front, improving resolution, and synchronizing migration through the gel.

Visualization of Proteins

As with DNA gels, there are several dyes used to label proteins in gels. Coomassie stain is arguably the most popular stain. The simplest Coomassie stain recipe requires brilliant blue R250 dye at a final concentration of 0.008%, HCl at final concentration of 35-50 mM. Gels can be stained with the dye for up to 3 hours and destained with water up to several days. More commonly, researchers use 0.1 % brilliant blue R250, 50 % methanol, and 10 % acetic acid. But similar to the movement away from ethidium bromide, researchers are beginning to use less toxic methods for protein analysis.

Imaging on the UVP GelSolo with the Biotium 1kb DNA ladder

Sample Preparation and Running Condition

The UVP GelSolo is equipped with epi-white light illumination to assist with protein gel images. In addition, white light converter plates are available for the GelSolo as an accessory feature. Mouse serum samples were quantified using the Bradford Assay and measured on an Analytik Jena Specord 250 UV/Vis spectrophotometer. Samples were serially diluted 1:2 and loaded on to a 4-12 % Bolt Bis-Tris Plus gel from Invitrogen (Waltham, MA). From left to right, total protein per lane ranges from 20 µg to 39 ng. Prior to loading, samples were solubilized 1:2 in 2x Laemmli buffer from Bio-Rad (Hercules, CA) and boiled at 85 °C for 5 minutes. The gel was run at 200 V (constant voltage) for 32 minutes per the manufacturer's instructions. The gel was rinsed 3 x 5 minutes in milliQ water to remove salt and excess detergents that may interfere with the protein stain. The gel was stained for 1 hour with SimplyBlue Safe Stain from Invitrogen (Waltham, MA) and destained for 3 hours with milliQ water.

Image Capture Conditions

The gel image in Figure 1 was captured using the UVP GelSolo. Exposure time was set to 22 ms. The histogram adjustment was set to auto. A UV transilluminator was used for excitation at 302 nm with a white light converter plate. Although we did not use the maximum sensitivity protocol from the manufacturer, we were able to reach near the limit of detection of the dye (i.e. 20 ng) using the basic protocol since lane 10 represents a total of 39 ng with 3 visible bands. This sensitivity is a testament to the quality and robustness of Analytik Jena's line of advanced imaging systems.

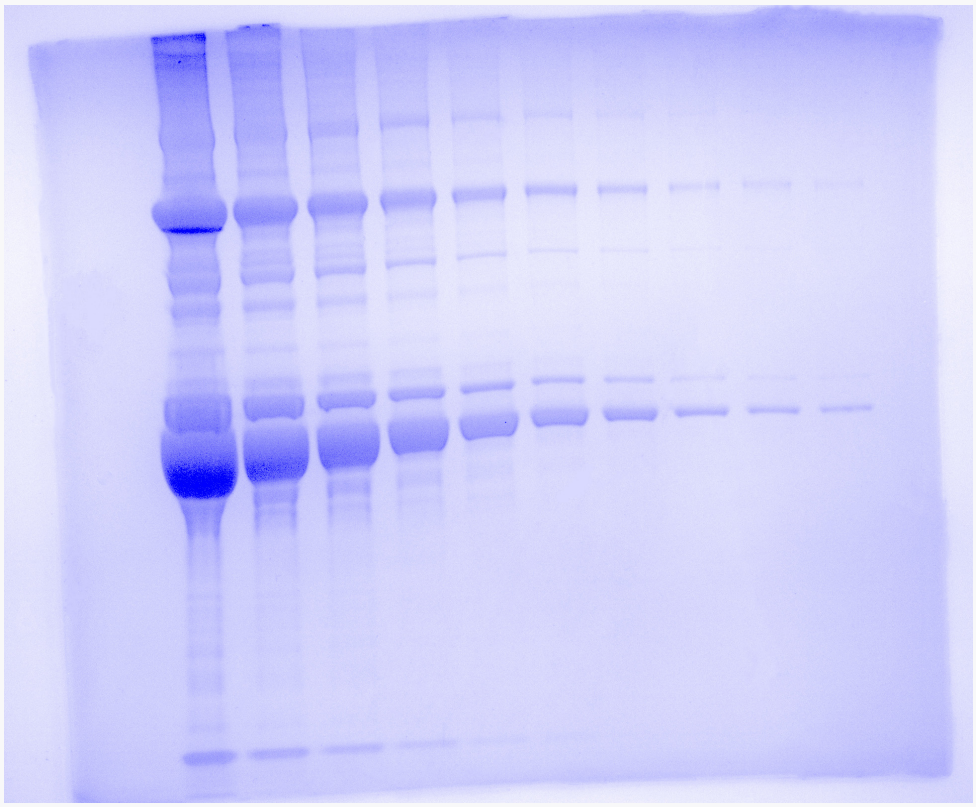

Figure 1: One dimensional SDS polyacrylamide gel electrophoresis using the UVP GelSolo. Mouse serum was run on the gel in 1:2 dilution series from 20 µg to 39 ng on a 4 - 12 % Bolt Bis-Tris Plus Gel for 32 minutes at 200 V. Gel was stained with SimplyBlue Safe Stain and imaged on the UVP GelSolo with 302 nm UV light and a white light converter plate with an exposure time of 22 s. Image was pseudocolored with Coomassie blue.

Expert Tips

- For extended electrophoresis runs, place the gel running tank in ice in a cold room to keep the buffer from overheating.
- Clean out wells with running buffer prior to loading samples to promote even migration of samples through the gel.
- Use a lab marker to outline wells on the gel so that loading samples into wells is easier.
- Dispense sample into well slowly so that samples aren't accidently forced out of the well.
- Cut costs by reusing running buffer at least once, but pay attention to overheating.
- Add the same volume of 1X Laemmli buffer to your samples, to your empty wells, to avoid anomalous migration patterns.
- Avoid loading samples or ladder in end wells to avoid edge effects.
- Vortex samples before and after heating to promote homogeneity of solubilization.
- Spin down samples after heating and vortexing, as some of the sample may evaporate and condense on the tube lid, therefore changing sample concentration.
- Do not overload wells. Do not exceed 20 µg for complex mixture of protein or 2 µg for purified protein.

References

1. Vickery, H. B. The Origin of the Word Protein. Yale J Biol Med 22, 387–393 (1950).

2. Pederson, T. Turning a PAGE: the overnight sensation of SDS-polycrylamide gel electrophoresis. The FASEB Journal 22, 949–953 (2008).

3. Reid, M. S. & Bieleski, R. L. A simple apparatus for vertical flat-sheet polyacrylamide gel electrophoresis. Analytical Biochemistry 22, 374–381 (1968).

4. Studier, F. W. Bacteriophage T7. Science 176, 367–376 (1972).

5. Laemmli, U. K. Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. Nature 227, 680 (1970).

Analytik Jena US LLC
2066 W. 11th Street
Upland, CA 91786 · USA

Phone +1 909 946 3197
Fax +1 909 946 3597

info@us.analytik-jena.com
www.us.analytik-jena.com

en · 01/2019