Water Makes the Difference

Ultrapure water improves results in 2D gel electrophoresis

Are there differences in quality between stored and freshly prepared ultrapure water? This article presents the results for the use of ultrapure water in gel electrophoresis and provides recommendations for the production of ultrapure water.

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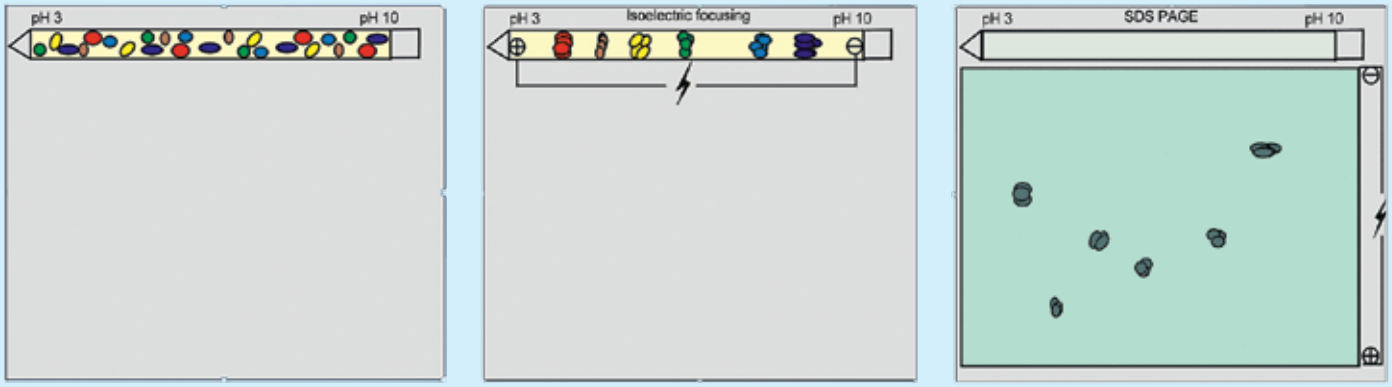

Source: Serva Electrophoresis GmbH, Heidelberg

1 Principle of 2D gel electrophoresis: Isoelectric focusing (IEF) is performed in the first dimension to separate proteins according to their isoelectric points (see center image). In the second dimension, proteins are separated in SDS electrophoresis (for SDS PAGE, see image on the right) according to their molecular weights.

Two-dimensional gel electrophoresis, called 2D PAGE for short, was developed by Klose and O'Farrell independently of each other in 1975 [1, 2]. It is used to separate protein mixtures into individual proteins and today has become an indispensable method in protein analysis (proteomics).

This 2D procedure consists of two separation principles, known as dimensions (see Fig. 1). While in the first dimension, ampholytes or immobilizing pH gradients (IPGs) are used to separate proteins according to their isoelectric points, in the subsequently second dimension, proteins are separated according to their molecular weights in SDS PAGE. Afterwards, the separated proteins are visualized using different staining methods, (Coomassie blue, silver staining, etc.) and employed to perform subsequent downstream analysis, as needed.

The quality of the results of a 2D gel electrophoresis is affected by many factors: The type of electrophoresis system used and the purity of the chemicals employed are decisive. While the advantages of using horizontal high performance electrophoresis (HPE) (see Fig. 3b) over a conventional vertical system have already been shown [3], this study examines the effect of water quality on silver staining.

Silver staining according to Blum et al. [4] was used to visualize proteins after 2D gel electrophoresis. This method is characterized by a very high sensitivity and places high requirements on the purity of the chemicals and ultrapure water used, which is reflected in the intensity and number of spots. The influence of water quality on silver staining was stud-

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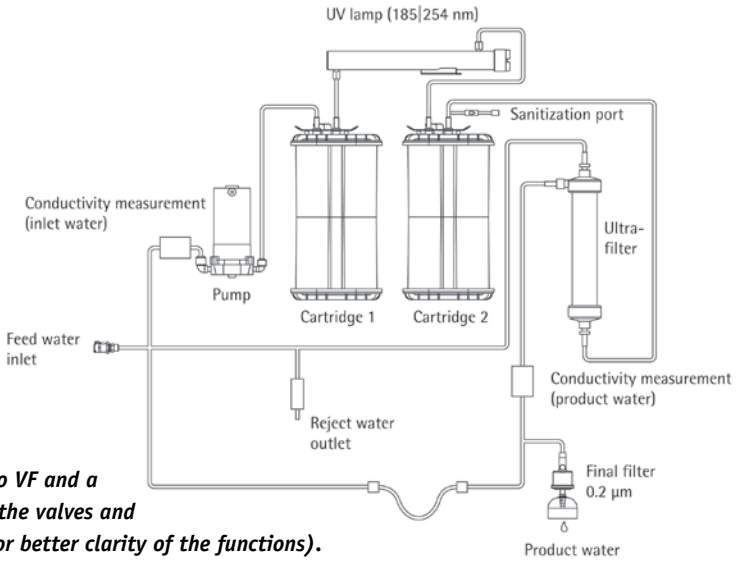
LP-TIP ■ Use of ultrapure water

In laboratory practice, it is often common to store ultrapure water in supply tanks or to use commercially available ultrapure water from glass bottles that may have been opened some time ago. Laboratory tests revealed that ultrapure water that has been left to stand in an open bottle has a much higher TOC and higher conductivity than does freshly dispensed ultrapure water (data not published). This is probably caused, inter alia, by transfer of volatile organic compounds and CO_2 from air into the water.



Source: Sartorius

2 Current model of the ultrapure water system Arium pro VF and a schematic flow diagram of the ultrapure water system (the valves and their controllers have been omitted from this diagram for better clarity of the functions).

ied. Two different grades of water were tested for their suitability:

- Ultrapure water produced by the Sartorius Arium pro VF water purification system and
- Water from the same system that had been stored for one week in a glass bottle (Erlenmeyer flask made of Duran glass and supplied by Schott; covered by perforated parafilm) at room temperature in a light place (referred to in this paper as "flask water").

The ultrapure water used was prepared as follows:

The Arium pro VF system (see Fig. 2) was used to generate ultrapure water for 2D gel electrophoresis. It removes impurities still present in pretreated tap water.

Production of ultrapure water requires continuous recirculation and a constant water flow, which is achieved by a pump system with pressure control. The conductivity of the water is measured at the feed water inlet and in the product water (ultrapure water dispensed directly from the water outlet).

Used in the studies described in this paper, the Arium pro VF is a predecessor model with identical technical specifications for production of ultrapure water as the redesigned system shown above, and works with two different cartridges. These are filled with a special active carbon absorber and mixed bed ion exchange resins in order to deliver ultrapure water with a low TOC content (total organic carbon). In addition, a UV lamp is integrated into the system, which has oxidizing and germicidal effects at wavelengths of 185 nm and 254 nm.

Furthermore, the Arium pro VF system has a built-in ultrafilter module used as a crossflow filter. The ultrafilter membrane it employs retains colloids, microorganisms, endotoxins, RNA, DNA and RNases, which is essential for the 2D gel electrophoresis runs performed and for subsequent silver staining. A 0.2 µm final filter installed on the water outlet serves to remove particles and bacteria from the ultrapure water produced. The process of water purification specifically performed by this system is shown in Figure 2 (flow diagram of Arium pro VF).

Materials and Methods

The materials used (chemicals and equipment) were supplied by Serva Electrophoresis GmbH, Heidelberg, Germany, if not otherwise specified.

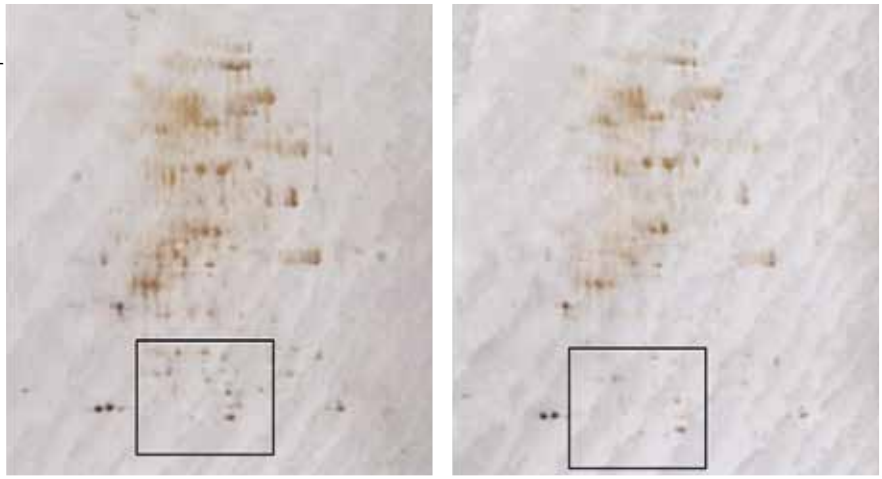
Escherichia coli (E.coli) extract: E.coli extract was prepared by two freeze-thaw

Source:Hoefer Inc.

Source:Serva Electrophoresis

3 Hoefer Inc. IEF100 (a) and HPE BlueHorizon (b) with Power Supply BluePower 3000

Source: Serva Electrophoresis



4 Silver staining of the gels: left, Sartorius Arium pro VF water; right, "flask water." The marked area shows the regions whose densitograms were compared (see Fig. 5).

cycles of lyophilized E. coli cells (ATCC 11303, Sigma-Aldrich Chemie, Munich, Germany) in 9 M urea, 65 mM dithiothreitol (DTT), 4% (w/v) 3-[(3-cholamidopropyl)dimethylammonio]-1-propansulfonate (CHAPS), 0.8% (v/v) Servalyt 3-10.

2D gel electrophoresis and silver staining: Isoelectric focusing (first dimension) was performed with Serva IPG Bluestrips, with a length of 11 cm, at a pH 4 – 7 in a Hoefer IEF 100 IPG focusing chamber (see Fig. 3a). For this purpose, the IPG strips were rehydrated overnight with 100 µg E.coli extract in 190 µL rehydration solution (8 M urea/0.5% CHAPS/0.2% DTT/0.5% Servalyt 3-10(v/v)) per strip.

Afterwards, focusing was performed using the following program:

Current per IPG strip: 75 µA, 250 V 1 h; 500 V 1 h; 5,000 V 30 min and 5,000 V to 27,000 Vh.

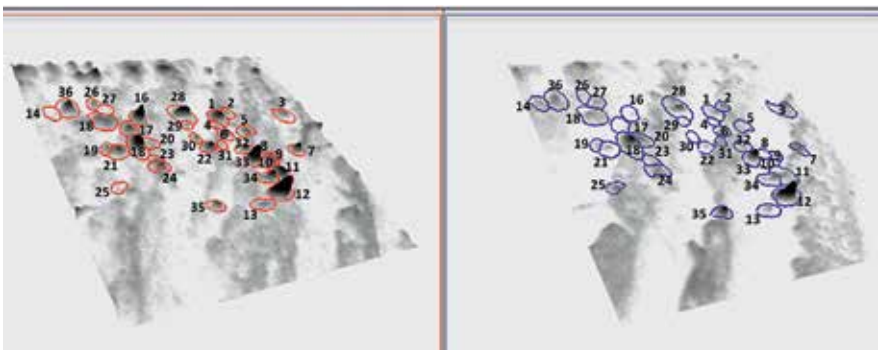
Before SDS PAGE (second dimension) was carried out, each of the focused IPG strips was equilibrated for 15 minutes in 30% glycerol, 6 M urea, 2% SDS, 50 mM Tris with a pH of 8.8, 1% DTT and 30% glycerol, 6 M urea, 2% SDS, 50 mM Tris with a pH of 8.8, 2.5% Iodacetamide.

SDS PAGE was run on a 2D HPE large format gel, 12.5%, in an HPE Blue Horizon chamber (see Fig. 3b). Both IPG strips were placed on a gel. Separation of the proteins was carried out under the following running conditions:

V = 100 V, I = 7 mA, P = 1 W, t = 30 min; V = 200 V, I = 13 mA, P = 3 W, t = 30 min; V = 300 V, I = 20 mA, P = 5 W, t = 10 min; V = 1,500 V, I = 40 mA, P = 40 W, t = 3 h 20 min

Following gel electrophoresis, the gel was halved. To visualize the protein spots, silver staining was performed according to Blum et al. [4]. All solutions needed for staining were prepared either using ultrapure water dispensed from the Arium pro VF system or with "flask water" to compare the effect of water quality on staining. The gels were fixed for 30 minutes in 40% ethanol and 10% acetic acid and washed three times with 30% ethanol for 10 minutes each time. Afterwards, one-minute pretreatment with a sodium thiosulfate solution, staining with a silver nitrate solution and development using a sodium carbonate/formaldehyde solution were performed. Silver staining (see Fig. 4) was stopped using a 1% glycine solution. The gels stained in this manner were evaluated using Delta 2D software supplied by Decodon (see Fig. 5).

Source: Decodon



5 Three-dimensional densitograms of the areas marked in Fig. 4. Left: fresh Sartorius Arium pro VF water; right: "flask water"

Results of Image Evaluation

"Flask water" (see Fig. 4, right) used in silver staining according to Blum resulted in significantly poorer spot intensities than did the freshly prepared ultrapure water (see Fig. 4, left), which is indicated by larger spot areas and weaker spot volumes. The number of spots of 348 remained nearly unchanged. As an example, the regions marked in Fig. 4 were compared with respect to spot areas and spot volumes. Thirty-six spots (see Fig. 5) were more closely examined. The spot ratio areas and volumes are depicted graphically in Fig. 6.

Spots silver-stained using solutions prepared with "flask water" have a 19% larger area on average (mean value of the area ratios of "flask" to Arium water) than the spots stained with solutions prepared with Arium water.

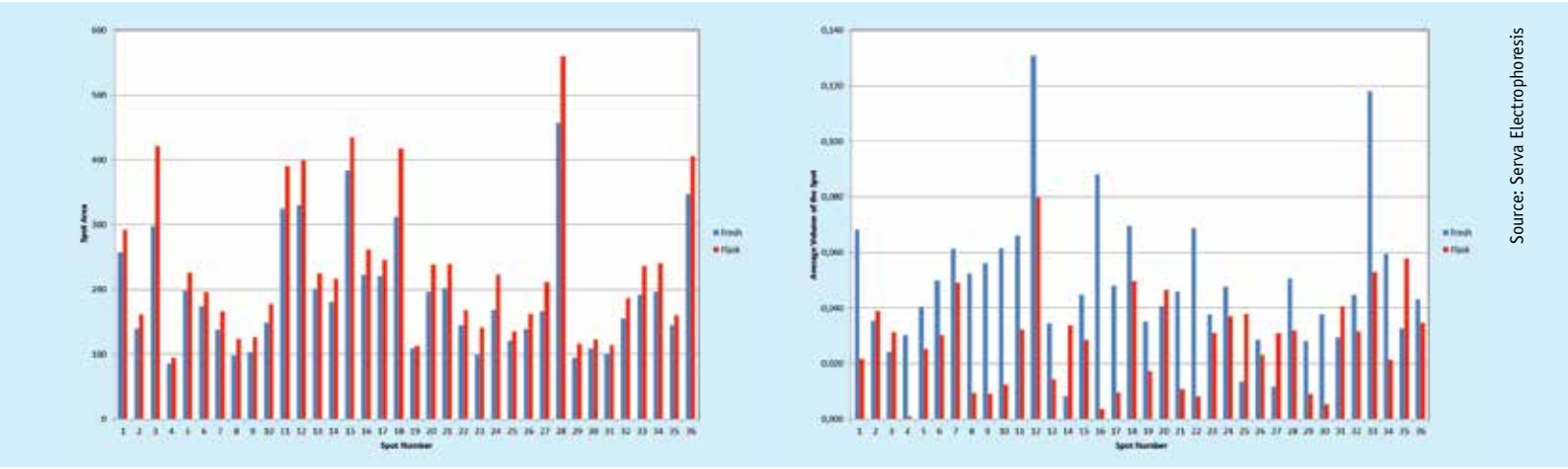
A larger spot volume (average gray scale) was found in 89% of the silver-stained spots prepared with fresh ultrapure water (32 out of 36).

This means that the spots silver-stained with solutions prepared using "flask water" are more diffuse on the whole and have a lower protein quantity per area. It is also apparent that approx. 20% of the spots either could not be stained at all or were difficult to stain with solutions prepared using "flask water" (spot volume <0.01).

Discussion

The effects that the use of stored ultrapure water can have are shown by the results of silver staining: the sharpness of the spots correlates with the purity of the water (for further comments, please see LP Tip).

Poorer water quality results in the decreased quality of silver-stained spots, which are diffuse, have a larger area and a less intensive stain. This signifies a



Source: Serva Electrophoresis

6 Graphic representation of area and volume of the spots marked in Fig. 5

lower protein quantity per area and is disadvantageous for quantification and subsequent analyses, e.g., if spots need to be excised using a spot picker to further process these gel plugs for subsequent mass spectrometry.

Poorer silver staining quality lets us conclude that the presence of TOC is increased. This deterioration in quality agrees with the results of Tarun et al. [5], who found that the water quality is decisive for the quality of protein separation in 2D electrophoresis. Gels and buffers prepared using "flask water" resulted in a lower number of spots; i.e., ultimately, fewer proteins could be identified.

The influence of increased TOC values needs to be examined in more detail in additional tests. The effect of other protein analysis methods, such as ELISA and Western blotting, must also be further studied.

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In conclusion, water of the best quality must always be freshly used for high-quality 2D electrophoresis and silver staining. ■

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