Afyon™ SDS-PAGE sample preparation kit

Fast and efficient concentration and cleanup of protein samples prior to gel electrophoresis or Western blotting

Frequently, dilute protein samples must be concentrated to enable visualization of bands of interest on a gel or western blot. The Afyon SDS-PAGE sample preparation kit provides a fast, efficient alternative to common methods such as acetone or TCA precipitation when protein samples must be concentrated prior to electrophoresis. In less than ten minutes, samples are ready to load on a gel, making Afyon convenient enough for routine applications.

The Afyon SDS-PAGE sample preparation kit provides a means to quickly concentrate protein samples, or to separate protein from buffers that interfere with electrophoresis.

Frequently, dilute protein samples must be concentrated to enable visualization of bands of interest on a gel or Western blot. Present options for concentration include TCA or acetone precipitation, or centrifugation using molecular weight cut-off membranes. TCA precipitation is time consuming, acetone precipitation requires large volumes, and the resulting protein pellet can be difficult to resuspend completely. Molecular weight cut-off spin filters can be problematic for very dilute solutions of protein since protein can be lost due to non-specific adsorption to the tube or filter.

If the downstream application for a concentrated protein sample is gel electrophoresis or Western blotting, Afyon provides a fast, efficient alternative. The Afyon protocol for concentrating dilute protein solutions is simple and takes less than 10 minutes (Figure 1). Essentially, Afyon resin is added to the dilute protein solution, the mixture is vortexed, the resin-bound protein is pelleted by centrifugation, and the protein is eluted from the resin by adding gel loading buffer that contains detergents or chaothropic agents. The resin is removed by using the spin filter included with the Afyon kit. The resulting sample is ready to be loaded on a gel.

In this Application note, we demonstrate the use of Afyon resin to concentrate a dilute solution of protein, allowing easy visualization on a Coomassie-stained gel and fluorescent Western blot.

Fast Protein Concentration

To demonstrate the efficiency of concentration using Afyon, a protein extract from A431 cells was diluted, and concentrated using the Afyon kit. Initially, the cell extract was adjusted to a concentration of 2 mg/ml; protein bands in 10 µg of this extract are easily visualized after electrophoresis using Coomassie R-250 staining (Figure 2, lane 2).

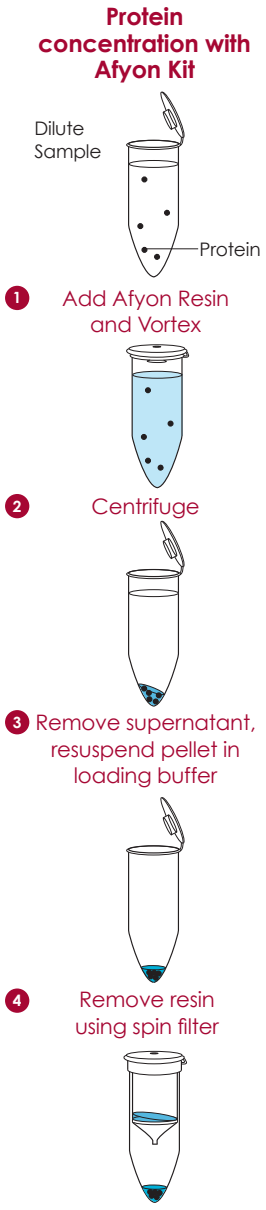

Figure 1. Protocol for protein concentration using the Afyon kit. Afyon resin is added to a dilute protein solution and vortexed. After centrifugation, the supernatant is removed from the resin pellet, and the pellet is resuspended in SDS-PAGE loading buffer. The resin is removed by using the spin column provided with the Afyon kit. The resulting sample is ready to load on a gel.

Then, 10 µl of the A431 protein extract was diluted 100-fold by the addition of 1 ml of PBS. When staining with Coomassie R-250, no bands can be seen when approximately 100 ng of protein is loaded on the gel (Figure 2, lane 3).

20 µl Afyon resin was added to the diluted protein solution, and the mixture was vortexed for 30 seconds. The protein, now bound to the resin, was pelleted by centrifugation at 10,000 x g for 1 minute. 950 µl of supernatant was removed by pipetting, and the pellet was centrifuged again for 1 minute. The remaining supernatant was removed, and the pellet resuspended by adding 10 µl of 2X Laemmli SDS-loading buffer and vortexing for 30 seconds. The mixture was added to a spin filter, and spun for 1 minute at 10,000 x g. 11 µl of the filtrate was recovered. The concentrated protein solution was heated at 95 °C for 5 minutes and loaded on the gel (Figure 2 lane 4).

The amount of the concentrated protein obtained with the above procedure is sufficient for one lane of a mini-gel. It can now be easily visualized by Coomasie staining (Figure 2, lane 4), and the banding pattern is virtually identical to that of the original sample (Figure 2b). When spin filters are used to remove the resin, some of the protein will remain in the "dead" volume of the spin filter. This protein can be recovered using additional washes, though the subsequent eluates will be less concentrated than that collected from the initial spin.

The Afyon procedure is compatible with fluorescent Western blotting using WesternBright® MCF (Figure 3). The same material used in the Coomassie-stained gel (Figure 2) was run on a duplicate gel and transferred to PVDF membrane, blotted, and subjected to fluorescent detection following the recommended WesternBright MCF protocol. After dilution, bands were not detectable (Figures 3a and 3b, lanes 3), while after concentration with Afyon (Figures 3a and 3b, lanes 4), bands are visible and identical to those in the original, undiluted sample (Figures 3a and 3b, lanes 2).

In conclusion, Afyon is a fast, easy way to concentrate dilute protein samples prior to electrophoresis and Western blotting.

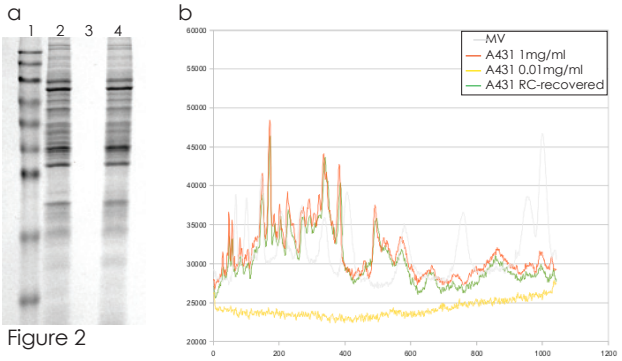

Figure 2

Figure 2. Effective concentration of a protein sample using the Afyon kit. Figure 2a: Coomassie R-250 stained gel comparing initial A431 cell extract (lane 2), diluted cell extract (lane 3), and concentrated sample after the Afyon protocol (lane 4). Lane 1 is molecular weight marker. Figure 2b: profile of lanes on the stained gel, demonstrating that the banding patterns of the initial and concentrated samples are identical.

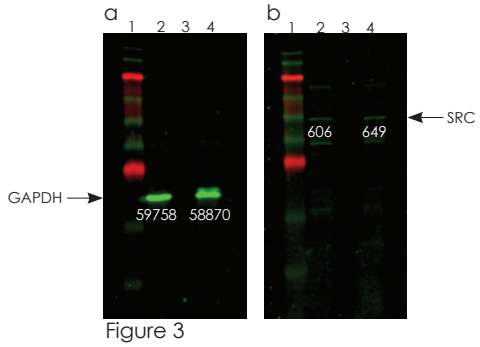

Figure 3

Figure 3. Afyon concentration is compatible with Western blot detection. The same samples used in Figure 2 were subjected to Western blotting followed by fluorescent detection using the WesternBright MCF multicolor fluorescent Western blotting kit. No bands were detectable in the diluted cell extract when stained for either GAPDH (Figure 3a lane 3) or SRC (Figure 3b lane 3). However, after Afyon concentration, bands are easily visualized for both proteins (Figures 3a and b, lanes 4). The band intensities are indicated.

Catalog Number	Product	Size
K-02101-010	Afyon™ Kit	10 RXNS
K-02101-025	Afyon™ Kit	25 RXNS
K-12021-010	WesternBright® MCF fluorescent Western blotting kit	10 blots

Advansta Corporation
2140 Bering Drive | San Jose, CA 95131
Tel: 650.325.1980 | Fax: 650.325.1904 | Email: sales@advansta.com
Product information: www.advansta.com/products/Afyon