5. Remove the supernatant. If using micro-centrifuge tubes, remove the supernatant as completely as possible, making sure not to disturb the blue pellet of the resin. If the total volume is too large for a micro-centrifuge tube, remove the supernatant in two steps. Refer to the details on the right.	Using large initial sample volumes and 15 ml or 50 ml tubes, remove most of the supernatant, and leave 50 to 500 µl the resin/sample mix in the tube. Pipette this mixture up and down until the resin suspension is uniform and transfer it to a micro-tube. Spin down this mixture in a micro-centifuge at maximum speed for 30 seconds and remove all the supernatant making sure not to disturb the blue pellet of the resin.
6. Add extraction-loading buffer (non-reducing) to the pellet. Use 15 µl of sample buffer per each 20 µl of Afyon resin added to the initial protein solution in step 2.	Extraction-loading buffer (non-reducing) is included with the Afyon resin kit.
7. Vortex the mixture for 1 minute.	
8. Transfer the mixture of the buffer with Afyon resin into the spin filter insert of the filtration device included in the kit.	
9. Centrifuge the spin filtration device at maximum speed (14,000 rpm) for 1 min to filter out the resin.	
10. Discard the filter insert with used resin.	Do not re-use Afyon resin.
11. The resulting collected filtrate is ready to load on an SDS-gel.	

Warranty

This product is warranted to be free of defects of material or workmanship, and to perform as described in the published specifications when stored according to the documentation included with the product, and used according to the accompanying instruction manual by appropriately trained personnel. If the product is found to have a defect upon first use and within 30 days of shipment, the product may be replaced. This warranty extends only to the original purchaser of the product. There is no obligation to replace the product as a result of misuse, improper storage, or negligence of the buyer.

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Afyon™

Fast, easy concentration and purification of protein samples for SDS-PAGE

For Catalog Numbers

K-02101-010	Afyon SDS-PAGE Sample Preparation Kit, 10 samples
K-02101-025	Afyon SDS-PAGE Sample Preparation Kit, 25 samples

Important Information

The following instructions are for use with the Afyon resin SDS-PAGE Sample Preparation Kit, catalog numbers K-02101-010 and K-02101-025.

The optimal pH range for protein binding to Afyon resin is 5.0 to 8.3, and optimal salt concentration is below 0.3 N.

Afyon resin is compatible with solutions containing the following commonly used reagents: Glycerol up to 10%, Ethanol up to 10%, Triton X-100 up to 0.1%, Tween-20 up to 0.1%, SDS up to 0.02%, Urea up to 0.5M, Guanidine Hydrochloride up to 0.05M.

Storage Information

Afyon resin is supplied as a 25% slurry in water. For long term storage, keep the tube containing the resin upright in a rack at 4° C. Do not freeze, boil, or autoclave the resin. Protect the resin from long exposure to bright light, and do not allow the resin to dry.

Warnings and Precautions:

- Afyon resin is for research use only.
- Always wear gloves when handling reagents.
- Refer to MSDS for additional safety information.
- The product is guaranteed to be free of manufacturer defect, and to function as described when the enclosed protocol is followed by properly trained personnel. Please see the Warranty section for more information.

Kit Contents

Catalog Number: K-02101-010

Afyon SDS-PAGE Sample Preparation Kit, 10 samples

Item	Description	Quantity
R-14012-500	Afyon Resin	500 µL
L-07002-010	Spin filters with collection tubes	10
R-03044-B10	Afyon extraction-loading buffer (non-reducing)	1 mL

Kit Contents continued

Catalog Number: K-02101-025

Afyon SDS-PAGE Sample Preparation Kit, 25 samples

Item	Description	Quantity
R-14012-500	Afyon Resin	2 X 500 µL
L-07002-025	Spin filters with collection tubes	25
R-03044-B10	Afyon extraction-loading buffer (non-reducing)	1 mL

Protocol

Step	Notes
1. Vigorously vortex the tube to re-suspend Afyon resin to homogeneity.	The resin settles quickly. If multiple samples are to be treated, vortex the resin frequently to make sure that suspension is uniform.
2. Add 20 µl of Afyon resin to the protein solution. This amount will be sufficient to obtain a protein sample for one lane of a typical mini-gel (~5 µg of total cellular protein). If more than one identical protein lane is required, increase the volume of Afyon resin accordingly.	Use regular pipette tips. Do NOT use very thin or narrow tips which may trap the resin. If the sample is very dilute, be sure to use a starting volume that contains sufficient protein for your subsequent application (e.g. approximately 5 µg of the total protein for electrophoresis.
3. Vortex the mixture for 30 seconds if the dilution factor for the resin is 1:100 or less.	For higher dilution factors (e.g. 1:1,000 to 1:10,000) increase vortexing time up to 5 minutes. An orbital shaker can be used for large volumes. Increasing incubation time longer than 5 minutes does not provide any advantage.
4. Centrifuge to pellet the resin.	30 seconds is sufficient for micro-tubes at maximum speed. For larger tubes, such as 15 ml or 50 ml conical tubes, 2 to 5 minutes at 3000g is sufficient.