

**ab133414 –
Optiblot SDS-PAGE
Sample Preparation Kit**

Instructions for Use

For fast, easy concentration and purification of protein samples for SDS-PAGE.

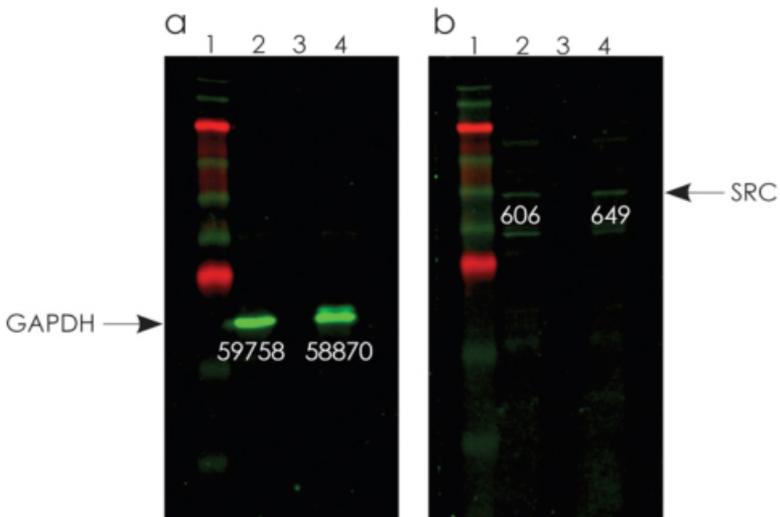
This product is for research use only and is not intended for diagnostic use.

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1. Introduction

The Optiblot SDS-PAGE Sample Preparation Kit (ab133414) provides a means to quickly concentrate protein samples, and separate them from buffers that interfere with electrophoresis. The fast, efficient protocol generates samples ready to load on a gel in less than ten minutes, much more quickly than can be achieved with alternate methods such as dialysis, or acetone or TCA precipitation. Additionally, the protocol is easily scaled up, allowing multiple samples to be prepared in parallel.



Resin concentration is compatible with Western blot detection. Western blots were created with samples of A431 cell extract (panels a and b, lane 2), diluted cell extract (lane 3) and the diluted extract concentrated using the Optiblot SDS-PAGE Sample Preparation Kit protocol (lane 4). The blots were detected using the Optiblot Fluorescent Western Blot Kit (ab133410). No bands were detectable in the diluted cell extract when stained for either GAPDH (panel a, lane 3) or SRC (panel b, lane 3). However, after resin concentration, bands are easily visualized for both proteins (lanes 4). Band intensities are indicated.

2. Assay Summary

Add 20 μ l of resin to sample
to obtain enough protein for one lane of a typical mini-gel



Centrifuge
to pellet the resin



Re-suspend pellet
in extraction loading buffer



Filter mixture
using spin filter insert



Collect filtrate
to load onto SDS-gel

3. Kit Contents

The Optiblot SDS-PAGE Sample Preparation Kit contains sufficient materials and supplies to purify 10 or 25 protein samples. The following components are included:

Item	Quantity (10 samples)	Quantity (25 samples)
Resin	500 mL	2 x 500 mL
Protein filters	10	25
Extraction loading buffer	1 mL	1 mL

4. Storage and Handling

Resin is supplied as 25% slurry in water. For long term storage, keep the tubes 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.

5. Protocol

1. Vigorously vortex the tube to re-suspend resin to homogeneity.

Note:

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 μ l of resin to the protein solution. This amount will be sufficient to obtain a protein sample for one lane of a typical mini-gel (\sim 5 μ g of total cellular protein). If more than one identical protein lane is required, increase the volume of resin accordingly.

Note:

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 μ g 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.

Note:

For higher dilution factors (e.g. 1:1,000 to 1:10,000) increase vortexing time by 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.

Note:

30 seconds is sufficient for micro-tubes at maximum speed. For larger tubes, such as 15 ml or 50 ml conical tubes, 2-5 minutes at 3000 x g is sufficient.

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.

Note:

Using large initial sample volumes and 15 ml or 50 ml tubes, remove most of the supernatant, and leave 50 to 500 μ l 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-centrifuge 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 to the pellet. Use 15 μ l of extraction loading buffer per each 20 μ l of resin added to the initial protein solution in step 2.

Note:

Extraction loading buffer is included with the Optiblot SDS-PAGE Sample Preparation Kit.

The Extraction-loading buffer is NON-reducing. For reducing conditions please add 2-mercaptoethanol to 5% (5 μ l per 100 μ l buffer)

7. Vortex the mixture for 1 minute.
8. Transfer the mixture of extraction loading buffer with 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.

Note: Do not re-use resin.

11. The resulting collected filtrate is ready to load on an SDS-gel.

Notes:

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

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.

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