EzWay™ PAG Protein Elution Refill Kit

1. Catalog No. K33015

2. Quantity 10 Tests

3. Introduction

EzWay™ PAG Protein Elution Kit is used to extract and collect proteins from denaturing polyacrylamide gel after SDS-PAGE separation. This kit provides a convenient and efficient method for homogenizing gel bands directly in a micro-tube using a simple device. EzWay™ PAG Protein Elution Refill Kit provides the pestle set, spin filter and elution buffer only for the researcher who already has a Mini Homogenizer.

Note: If you don't have a Mini Homogenizer, you have to order EzWay™ PAG Protein Elution Kit (K33011) at first. (Mini Homogenizer is not sold separately.)

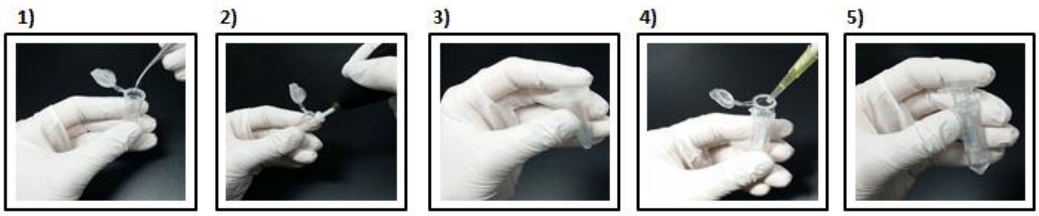
4. Kit Contents

Component	Size
Homogenizing Pestle Set (Pestle with 1.5ml micro-tube)	10 set
Spin filter	10 ea
Elution buffer	25 ml

5. Storage & Stability Store at room temperature

6. Protocol

Protocol at a glance



6) Place the gel slice in a 1.5ml micro tube.
7) Homogenize the gel slice using a pestle & mini homogenizer.
8) Add the Elution buffer to the tube.
9) Transfer the sample into a spin filter and centrifuge.
10) Eluted protein solution is collected in the collection tube.

Step A. Gel Slice Homogenization

5. Excise the interested band from the polyacrylamide gel.
6. Place the gel slice in a micro-rube.
7. Attach the pestle to the mini homogenizer, and place it in a tube.
   (The recommended amount of the gel slice per assay is 100 ul - 400 ul. It should be at least 100 ul. The maximum amount is 400 ul.)
8. Push the start button on the device until the gel slice is completely crushed. When the gel is homogenized, add two volumes of elution buffer to the tube. (If the gel slice is 100 ul, add 200 ul elution buffer.)

Note:

1. Elution buffer should be added after homogenization to avoid making bubbles during homogenization.
2. Gels should be stained with non-fixative method.

Step B. Protein Elution

8. Remove the pestle & homogenizer assembly from the tube, and vortex it for 5 min.
9. Transfer the homogenized sample to a spin filter. (The maximum capacity of the Spin filter is 300 ul.)

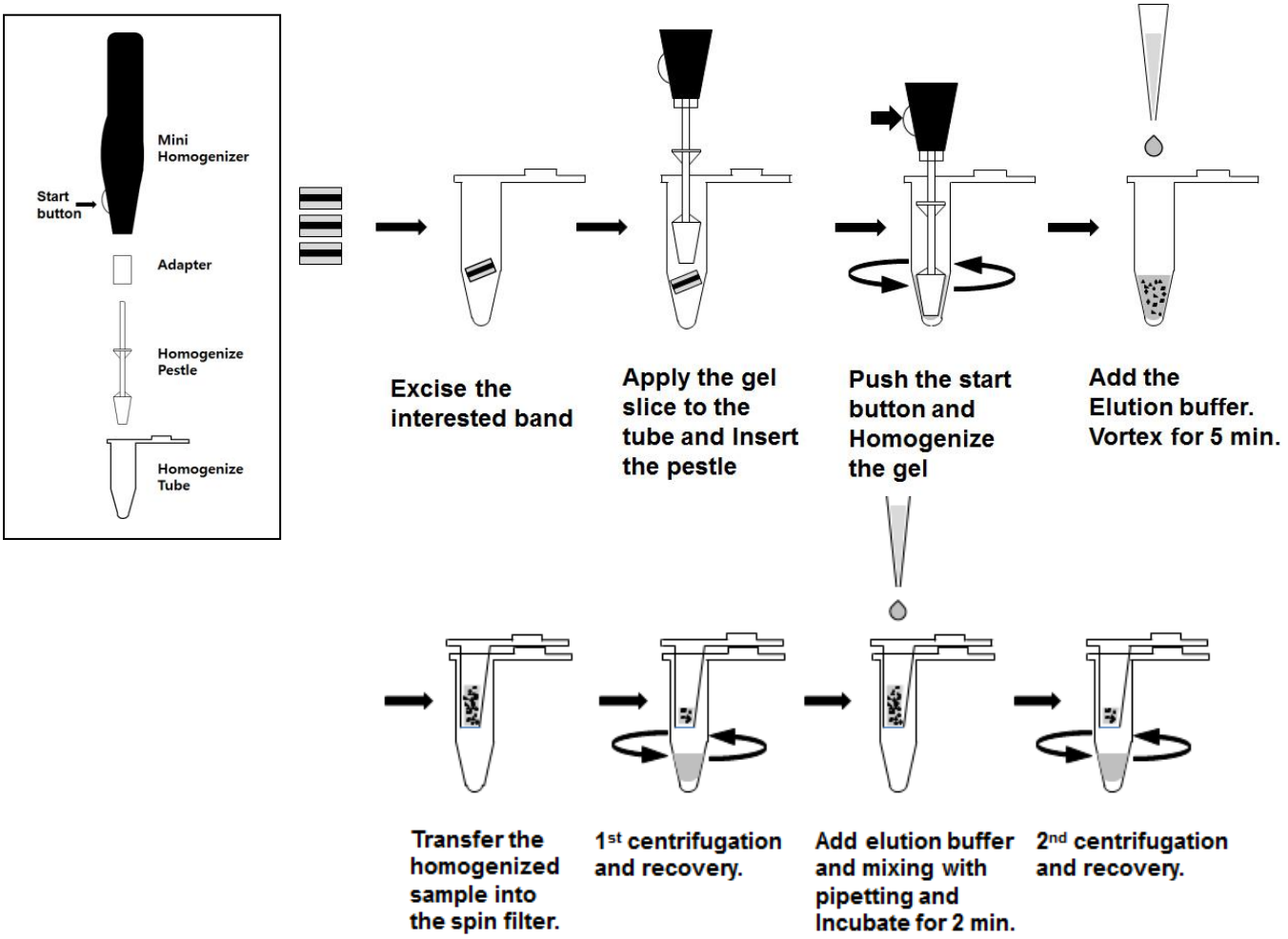
   Note: When transferring the homogenized slurry to the spin filter, pipetting might be difficult because this solution is sticky. You can use a cut tip (a pipet tip cut with a razor blade) or a micro spatula to take out the slurry.

10. Centrifuge (10,000 x *g*) for 3-5 min at room temperature or 4°C. Eluted protein solution is collected in the 2 ml collection tube by passing through the spin filter membrane after centrifugation.

    Note: The maximum spin speed of the spin filter is 15,000g. Be careful not to exceed this speed. Over speed might cause some breakage of the filter during centrifugation.

11. Transfer the eluted protein solution to a clean collecting tube.
12. For complete elution, add the equal volume of elution buffer to the spin filter. (If the mashed sample is 100 ul, add 100 ul elution buffer.)
13. Gently pipette up and down the mixture of sample and elution buffer in the Spin filter, and then incubate for 2 min at room temperature.
14. Repeat step 3 and 4.

    Note: If the color remains in the gel, repeat the step 5 and 6 until the residual color in the gel disappears. If the volume of the eluted protein solution is too much, then concentrate it using a concentrator.



7. Efficiency

Elution efficiency: > 70%
(It was determined from BSA running gel after 10% SDS-PAGE. But the elution efficiency varies according to the type of protein.)

8. Application

Recovery of the interested protein molecules after SDS-PAGE for preparing antigens
Notice : The Elution Buffer provided in this kit contains high concentration of detergent. so it is not suitable for MASS spectrometry assay purpose.

9. Troubleshooting

Problem	Cause	Solution
No band after elution	The protein concentration in the gel slice is too low.	The protein concentration has to be more than 4 ug per band.
	Due to incomplete elution, the protein might remain in the homogenized sample with gel slurry.	The blue color means the coomassie stained protein, so please repeat the elution step until the blue color in the gel (spin filter) is disappeared.