

# EzWay™ PAG Protein Elution Kit

**1. Catalog No.** K33010

**2. Quantity** 10 Tests

**3. Introduction** EzWay™ PAG Protein Elution Kit is used to extract and purify proteins from denaturing polyacrylamide gel after SDS-PAGE separation. It uses a simple device (masher bar and masher tube) for homogenization. Instead of the conventional electro elution method, the interested protein can be recovered easily, rapidly and conveniently with EzWay™ PAG Protein Elution Kit without any special equipment requirements. Only a table-top microcentrifuge is required.

**4. Kit Contents**

Component	Size	Cat. no.
Homogenizer (Masher tube, Masher bar, 2ml collection tube)	10 set	K330101
Spin filter	10 ea	K330102
Elution buffer	25 ml	K330103

**5. Storage & Stability** 1 year at room temperature

**6. Protocol**



1. Apply the gel slice to the masher tube.
2. Centrifuge. The mashed samples are collected in the collection tube.
3. (Optional) Elution buffer & extra clean gel slice minimizes the retention of the protein in the masher.
4. Transfer the sample into a Spin filter and Centrifuge.
5. Eluted protein solution is collected in the collection tube.

**Step A. Band Homogenization**

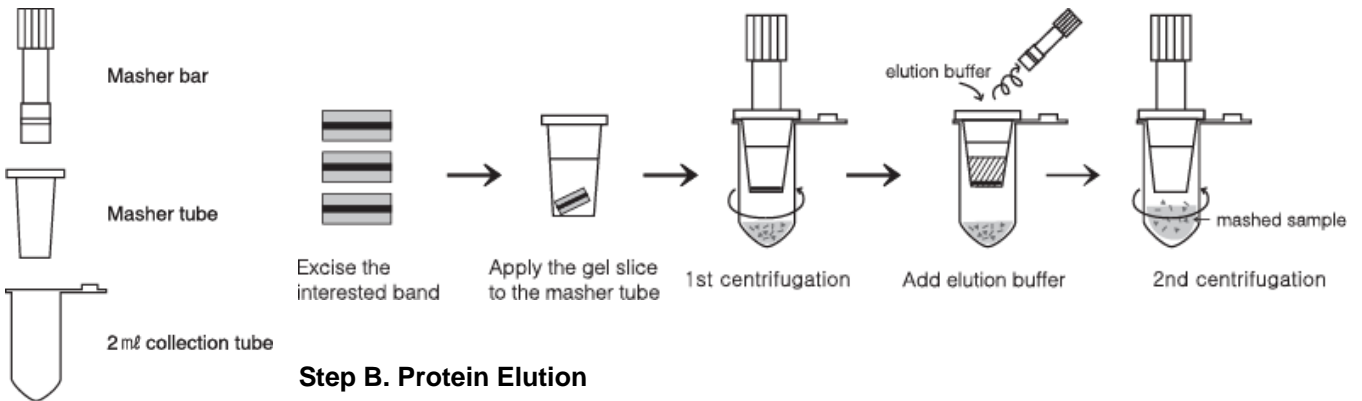
1. Excise the interested band from the polyacrylamide gel.
2. Place a masher tube in a provided 2 ml collection tube.
3. Apply the gel slice to the masher tube.
4. Place the masher bar into the masher tube. (The recommended amount of the gel slice per masher tube is 100 ul - 400 ul. It should be at least 100 ul. If the amount of the gel slice is not sufficient, add extra gel slice to make this volume. The maximum amount is 400 ul.)
5. Centrifuge (10,000 x g) the homogenizer for 2 min at room temperature or 4°C. (If the refrigerated microcentrifuge is available, centrifugation at 4°C is ideal to keep protein activity.)
6. Add the equal volume of elution buffer to the masher tube. (If the gel slice is 100 ul, add 100 ul elution buffer.)
7. Repeat step 4 and 5.
8. The mashed samples are collected in the collection tube.

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**Note:**

1. We recommend to put extra clean gel slices to fill up the space between masher and masher tube. In this case, add small pieces over several times rather than large piece at a time.
2. When adding the extra gel slices to the masher, the layer of the gel slice containing protein band should be placed at the very bottom.
3. Put the Masher bar into the Masher, so that it is helpful for better homogenization.
4. Usually for the staining of the gel for elution purpose, using non-fixative protein staining solution is recommended.



**Step B. Protein Elution**

1. Remove the masher tube, and then vortex the 2 ml collection tube that contains the mashed sample for 5 min.
2. Transfer the mashed sample from 2 ml collection tube into a Spin filter. (The maximum capacity of the Spin filter is 300 ul.)

**Note:** When transferring the mashed slurry to the spin filter it might be difficult to pipetting 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.

3. 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.

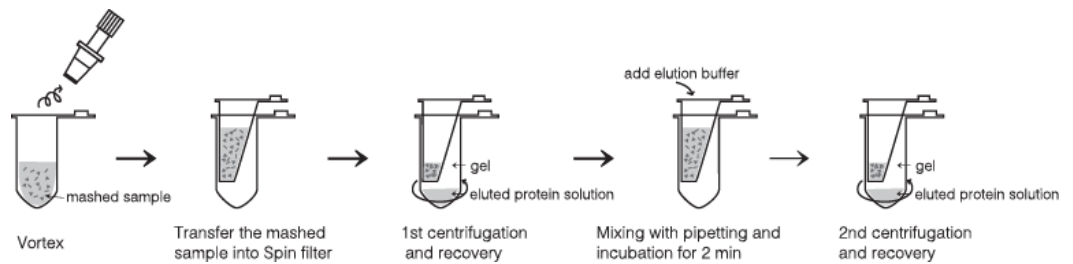
**Note:** The maximum spin speed of the spin filter in this kit is 15,000g. Be careful not to exceed this speed. Over speed might cause some breakage of the filter during centrifugation.

4. Transfer the eluted protein solution to a clean collecting tube before step 5.
5. 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.)
6. 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.
7. Repeat step 3 and 4.

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

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**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

**9. Troubleshooting**

Problem	Cause	Solution
<b>No band after elution</b>	The amount of the gel slice which was applied to the masher tube at the first step was not enough.	Please make sure the minimum amount should be at least 100ul. If the amount is not sufficient, extra clean gel slice has to be added to minimize the retention of the protein in the masher.
	The protein concentration in the gel slice is too low.	The protein concentration has to be more than 4ug per band.
	Due to incomplete elution, the protein might remain in the mashed 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.

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