Micro Protein PAGE Recovery Kit

Cat: G7200
Size: 20 Assays
Storage: All reagents are transported at room temperature. All reagents are stored at 2-8℃ after receipt. Solution B avoids fire sources. Solution A is easy to form precipitates at low temperatures. Please heat at 30℃ before use to promote dissolution. Valid for 1 year.

Product Composition:

Component	Size
Buffer A	10 mL
Buffer B	60 mL
Dry powder	9.6g
DTT	40 mg
Filter column	20
Collection tube	20

Completely dissolve the dry powder and DTT in solution A before use, store at 4°C after preparation, and heat at 30°C each time to promote dissolution. The mixed solution A should be used within one month as far as possible.

Product description:

SDS-PAGE can be used not only to detect the relative molecular mass of proteins, but also one of the important tools for separating and purifying proteins. With the miniaturization of protein technology, it is necessary to recover proteins from gels for the preparation of antibodies, immunoblotting, and amino acids component analysis or terminal sequence determination, etc. This product is a trace protein glue recovery method specially developed for this purpose. This product is suitable for recovering protein from Coomassie Blue staining, Cu staining or zinc staining. The recovery efficiency is 50-80%. This product can be used for 20 times.

Product features:

1. It is simple and does not require complex and expensive instruments (such as electric elution instrument).
2. Suitable for SDS-PAGE and Native-PAGE.
3. The recovery rate is generally between 50-80% (related to protein size and elution time).
4. Compatible with subsequent experiments, including 1-D, 2-D electrophoresis and mass spectrometry sequencing, etc.

Instructions for use(for reference only):

1. Use a scalpel to cut off the part of the Cu or Zinc stained gel containing the target band and put it into a 1.5 mL centrifuge tube. Use the corresponding elimination solution to decolor the protein band in the gel until the gel is close to colorless. Centrifuge at 12000 rpm (12830g) at room temperature for 5 minutes, try

to remove the supernatant and retain the gel.

For Coomassie Blue staining or other staining methods, directly cut off the part of the gel that does not need to be decolorized or that has been decolorized by the decolorizing solution. Put the gel into a 1.5ml centrifugal tube, wash it twice with double distilled water for 5min each time and then proceed to step 2.

2. Grind the colloid in the centrifuge tube into small pieces with a grinding rod, add 400μL of solution A, shake overnight (16-18 hours) on a decolorizing shaker at room temperature, take it out during the period, and shake it occasionally.

3. Pipette the mixture into the filter column with a 1mL tipless pipette tip (pipette the gel together and add the solution in several times if there is too much) and centrifuge at 13,000 rpm for 5 minutes. Transfer the filtrate in the collection tube to a new centrifuge tube, add 2 mL of pre-cooled solution B, mix well, and place it at 4℃ for 30 min.

4. Centrifuge at 12000 rpm at room temperature for 15 minutes, remove the supernatant, and place the centrifuge tube in a fume hood until the remaining liquid evaporates. You can also add 0.5 mL of solution B again, shake to wash the precipitate, place it at 4°C for 15 minutes, and repeat step 4 one or two times, so that the protein obtained is more pure.

5. Dissolve the precipitated protein with the suitable buffer after the liquid evaporates for subsequent experiments such as electrophoresis and mass spectrometry sequencing.

Note:

1. After ensuring that the gel containing target protein is completely cut, remove the excess gel as much as possible.

2. If the molecular weight of protein is large, the amount of sample added is large and the gel concentration is high, the incubation time can be appropriately prolonged.

3. In order to have a better recovery effect, the amount of the protein added in each sample hole is preferably between 2μg-40μg.

4. Using a dissolving solution containing strong solvency reagents such as Urea, Thiourea and SB3-10 helps to completely dissolve the precipitated protein.

5. When grinding the gel fragments, the more fully, the better, which is conducive to the diffusion of protein into solution A.

6. Silver staining is generally not recommended for gel recovery when using this kit because the recovery efficiency is low.

7. The recovery efficiency of the target protein has a certain relationship with the molecular weight of the protein. The recovery efficiency of the polypeptide below 10 KD is significantly reduced. If the molecular weight is greater than 120 KD, it is necessary to increase the overnight shaking time in step 2.

8. If the concentrated gel is prepared without adding the comb, the protein solution is spread evenly on the surface of the concentrated gel for electrophoresis, then a complete target protein band from left to right needs to be cut off for gel recovery. The amount of the reagents should be increased in multiples according to the the number of sample holes in the gel with a comb.

9. The volume of solution A and solution B needs to maintain a certain proportional relationship. The volume of solution B must be 5 times or more than that of solution A to avoid unnecessary precipitation of impurities in the process of step 3.

10. The amount of reagents added in the process is determined for the 80×60×1mm gel. If the gel volume is larger, the amount of each reagent can be increased appropriately.