

ab286925 – DiaEasy Dialyzer (3 ml) MWCO 12-14 kDa

For purification of very small quantities of proteins (up to 0.5 µg), for automated protein sequencing, peptide mapping and amino acid analysis.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab286925>

Storage and Stability

Store all components of the kit at room temperature.

Materials Supplied

Item	Quantity	Storage Condition
DiaEasy Dialyzer Tubes	10	Room temperature
DiaEasy Floating Rack	2	Room temperature
DiaEasy Supporting Tray	2	Room temperature

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Beakers
- Buffers

Δ Note: Read the entire protocol before performing the experiment.

Dialysis Protocol

1. Fill the DiaEasy Dialyzer Tube with 2-3 mL of dH₂O. Incubate for at least 5 min. Empty the tube.

Δ Note: Carefully check that no dH₂O leaking from the tube. Water absorption, by the dry membrane, causes decrease of water level.

2. Load sample into the DiaEasy Dialyzer Tube. Close the tube.

Δ Note: Sample volume should be in the range of 0.1-3 mL. If small volume is used, load the sample close to the inner membrane.

3. Place the loaded DiaEasy Dialyzer Tube in the supplied floating rack in a stirred beaker containing large volume (usually 100 to 1000-fold that of the sample) of the desired buffer. The floating rack can hold 1-7 DiaEasy Dialyzer Tubes. Adjust the stir bar speed. Allow at least 30 min for each 0.1 ml of sample. Low-molecular weight salts and buffers (e.g., Tris HCl and KPO₄) equilibrate within 3 hours. Equilibration times for viscous samples will be longer.

Δ Note: The user must determine exact equilibration times for the dialysis. Adjust the stir bar speed. Low-molecular weight salts and buffers (e.g., Tris HCl and KPO₄) equilibrate within 3 hours. Equilibration times for viscous samples will be longer.

4. Change the dialysis buffer as necessary.
5. Pipette out the sample carefully from the DiaEasy Dialyzer tube to a clean tube.

Δ Note: User must determine exact equilibration times for the dialysis.

Δ Note: If sample volume has increased during dialysis, let your sample evaporate on the bench top (a fan increasing airflow across the membrane will speed up the process), check every 10 min or less to prevent full evaporation and dryness.

Sample Concentration Protocol

1. Place sample in the DiaEasy Dialyzer tubes or use already dialyzed sample and place it on the microtube rack stand.
2. Let samples evaporate on the bench top (using fan to increase airflow across the membrane speed up evaporation process), check every 10 min or less to prevent full evaporation and dry sample condition. Concentrating by evaporation of water with sample, small molecules (buffer salts, reducing agents, etc.) will also get concentrated because no diffusion occurs.

Δ Note: When evaporating water from your sample, small molecules (buffer salts, reducing agents, etc.) will also be concentrated.

DNA/RNA Gel Extraction Protocol

1. Fill the DiaEasy Dialyzer tube with 2-3 mL of dH₂O. Incubate for at least 5 min. Empty the tube.

Δ Note: Check carefully that no dH₂O is leaking from the tube. Absorbent of water, by the dry membrane, cause to the decrease in water level.

2. Excise the slice of gel containing the desirable DNA or RNA fragment with a clean, sharp scalpel. Minimize the size of the gel slice by removing extra gel. Maximum gel slice size 2 cm x 1 cm.
3. Transfer the gel slice to a DiaEasy Dialyzer tube. Fill the tube with 2-3 ml dH₂O. Close the tube gently with DiaEasy Dialyzer 3 ml cap. Avoid air bubbles in the tube. Don't fill the tube with several gel slices, for larger gel slices use more than one tube.
4. Place the DiaEasy Dialyzer tube in the provided tray. The supporting tray can comprise 1-3 DiaEasy Dialyzer tubes.

Δ Note: The arrow on the cap is pointing face up. The two membranes of the DiaEasy Dialyzer tube must be in perpendicular to the electric field to permit the electric current to pass through the tube.

5. Place the supporting tray containing the DiaEasy Dialyzer tubes in a horizontal electrophoresis tank containing running buffer.

Δ Note: Immerse fully the DiaEasy Dialyzer tube with the tray in the buffer.

6. Pass electric current (usually at 100 volt) until the nucleic acid exits from the gel slice (see Tables 1 and 2).
7. Optional: Follow the DNA or RNA eluted out of the gel with a hand-held UV lamp or table.

Δ Note: The electro-elution time need to be adjusted for each individual sample.

8. Reverse the polarity of the current for 120 seconds. This step will release the nucleic acid from the membrane.
9. Open the DiaEasy Dialyzer tube gently, pipetting the solution up and down carefully (at least 5 times) and transfer the solution to a clean tube. Do the pipetting on the inner side of the membrane.

Δ Note: Concentrate the extracted nucleic acid by standard concentration methods.

Elution Timetables: In this method the elution time depends on the size of the nucleic acid fragment, the concentration of the gel, the size of the gel slice, the ratio of the polyacrylamide: bisacrylamide and the applied voltage.

Δ Note: The electro-elution time at the elution step needs to be adjusted for each individual sample.

Table 1: Minimum time needed to extract various DNA or RNA fragments from native or denatured 4% polyacrylamide gel (29:1 polyacrylamide: bisacrylamide) at 100 volts in 1XTBE buffer.

Fragment size (bp)	Elution time (min)
100	10-15
200	15-20
500	30-35
1000	55-60
1400	75-80

Table 2: Minimum time needed to extract DNA fragments from 1% agarose gel at 80-110 volt.

Fragment size (bp)	Elution time (min)
500	10-15
1000	15-20
2000	25-30
5000	40-45
8000	50-55
10000	55-60

DNA or RNA Precipitation Protocol

1. Add 0.1 volumes of 3 M Potassium acetate, pH-5.2 and 0.7-1 volumes of isopropanol to the solution. For example, add 0.3 ml of 3 M Potassium acetate pH-5.2 and 2.31 – 3.3 ml isopropanol to a 3 ml sample.
2. Mix gently by inverting the tube several times.

Δ Note: Addition of carrier (e.g., 80 μg tRNA or 80 μg glycogen) to the solution will increase the efficiency of precipitation.

3. Incubate at -20°C for 10 min.
4. To increase DNA or RNA precipitation yield incubate the samples overnight at -20°C.
5. Centrifuge the sample at 4°C for 30 min at 20,000 g.
6. Carefully discard the supernatant without disturbing the pellet.
7. Wash the pellet with cold 70% ethanol.
8. Centrifuge the sample at 4°C for 30 min at 20,000 g. Centrifuge the tube in the same orientation as previously to recover the DNA or RNA in a compact pellet.
9. Air-dry the pellet for 5-20 min.

Δ Note: Do not over-dry the pellet (e.g., by using a vacuum evaporator), as this will make the DNA, especially if it is of high molecular weight, difficult to redissolve.

10. Redissolve the DNA or RNA in a suitable buffer.

Δ Note: Use a buffer with pH >8.0 for redissolving, as DNA does not dissolve readily in acidic buffers.

Protein Extraction from Polyacrylamide Gel with DiaEasy Dialyzer tubes

Δ Note: Fixation of proteins before electro elution (e.g., fixation with methanol, acetic acid, etc.) is not recommended. Fixation greatly reduces extraction yield. A sensitive protein staining solution may be used, as it permanently stains the gel without undue fixing of the protein.

1. Fill the DiaEasy Dialyzer tube with 2-3 ml of dH₂O. Incubate for at least 5 min. Empty the tube.

Δ Note: Check carefully that no dH₂O is leaking from the tube. Absorbance of water, by the dry membrane, causes the decrease in water level.

2. After staining the gel, excise the gel slice containing the protein with a clean, sharp scalpel.
3. Minimize the size of the gel slice by removing extra gel. Maximum gel slice size should be 2 cm x 1 cm.
4. Transfer the gel slice to a DiaEasy Dialyzer tube. Fill the tube with protein-running buffer (2.5-3 mL). Close the tube gently. Avoid air bubbles in the tube. Do not fill the tube with several gel slices, for larger gel slices use more than one tube.
5. Place the DiaEasy Dialyzer tube in the provided supporting tray.
6. The supporting tray can hold 1-4 DiaEasy Dialyzer tubes.

Δ Note: The arrow on the cap should point face up. The two membranes of the DiaEasy Dialyzer tube must be in perpendicular to the electric field to permit the electric current to pass through the tube.

7. Place the supporting tray containing the DiaEasy Dialyzer tubes in a horizontal electrophoresis tank containing protein running buffer.

Δ Note: Immerse fully the DiaEasy Dialyzer tubes with the tray in the buffer.

8. Pass electric current (usually at 100 volt) until the protein exits from the gel slice.
9. Electro-elution time is to be adjusted for each individual sample. It takes at least 85 min for BSA protein to be electro-eluted from a 10% SDS-PAGE slice (see Table 3).
10. Reverse the polarity of the electric current for 120 seconds. This step will release the protein from the membrane.
11. Open the DiaEasy Dialyzer tubes gently, pipetting the protein-containing solution up and down carefully (at least 5 times) and transfer the solution to a clean tube.
12. Do the pipetting on the inner side of the membrane.

Δ Note: Use the extracted protein directly.

Δ Note: Concentrate the extracted protein by standard concentration methods.

Δ Note: Precipitate the extracted protein by standard precipitation protocols.

Δ Note: Dialyze directly the extracted protein with a clean DiaEasy Dialyzer tubes.

Elution Time: The elution time depends on the size of the protein molecule to be eluted, the applied voltage, the size of gel slice, the ratio of the polyacrylamide: bisacrylamide and the percentage of the polyacrylamide gel. Electro-elution time at the elution step was to be adjusted for each individual sample.

Table 3: Minimum time needed to extract different-sized proteins from 10% SDS-polyacrylamide gel (29:1 polyacrylamide: bisacrylamide) at 100 V, in 1XPB: 0.192 M Glycine, 0.025 M Tris-base and 0.1% SDS

Protein (kDa)	Time (min)
14	35-45
19-26	45-55
29	55-65
40	60-70
45	65-75
50	75-85
66	85-95
81	105-115
116	120-130
128	140-150

Protein Extraction from Polyacrylamide Gel Compatible with MALDI-MS Protocol

1. Fill the DiaEasy Dialyzer tube with 2-3 mL of dH₂O. Incubate for at least 5 min. Empty the tube.

Δ Note: Check carefully that no dH₂O is leaking from the tube. Absorbance of water by the dry membrane causes decrease in the water level.

2. After staining the gel, excise the gel slice containing the protein with a clean, sharp scalpel.

Δ Note: Minimize the size of the gel slice by removing extra gel. Maximum gel slice size should be 2 cm x 1 cm.

3. Transfer the gel slice to a DiaEasy Dialyzer Tubes. Fill the tube (0.7 to 0.8 ml) with protein running buffer containing 250 mM Tricine pH 8.5, 0.025% SDS and 25 mM Tris-Base. Close the tube gently.

Δ Note: Avoid air bubbles in the tube. Do not fill the tube with several gel slices, for large gel slices use more than one tube.

4. Place the DiaEasy Dialyzer Tubes in the provided supporting tray.

Δ Note: The supporting tray can hold 1-4 DiaEasy Dialyzer Tubes.

5. Place the supporting tray containing the DiaEasy Dialyzer Tubes in a horizontal electrophoresis tank filled with protein-running buffer: 250 mM Tricine pH 8.5, 0.025% SDS and 25 mM Tris-Base.

Δ Note: Immerse fully the DiaEasy Dialyzer Tubes with the tray in the buffer.

6. Pass electric current at 150 volts until the protein exits from the gel slice.

Δ Note: The electro-elution time is to be adjusted for each individual sample. It takes at least 2.5 hours for BSA protein to be electroeluted from a 10% SDS-PAGE gel slice in the size of 2 x 1 cm.

Δ Note: For other proteins from BSA, increase electro elution time presented in Table 3, by 30%.

7. Reverse the polarity of the electric current for 120 seconds. This step will release the protein from the membrane.
8. Open the DiaEasy Dialyzer Tubes gently, pipetting the protein-containing solution up and down carefully (at least 5 times) and transfer the solution to a clean tube.

Δ Note: Do the pipetting on the inner side surface of the membrane.

Protein Precipitation for Analysis by MALDI-TOF Protocol

1. Add 1:10 by volume of MS buffer of your choice to the protein containing solution and mix properly. For example, add 0.3 ml of MS buffer to a 3 ml sample.
2. Incubate for 15 min at room temperature.
3. Add 1: 5 by volume of 50% TCA and mix properly. For example, add 0.66 ml of 50% TCA to a 3.3 ml sample.
4. Incubate for 1 hour at 4°C.
5. Centrifuge the sample at 4°C for 30 min at 20,000 g.
6. Carefully discard the supernatant without disturbing the pellet.
7. Add 500 µl of ice-cold acetone.
8. Incubate at -20°C for 30 min and centrifuge the sample at 4°C for 30 min at 20,000 g.
9. To increase protein precipitation yield, incubate the samples overnight at -20°C.
10. Carefully discard the supernatant without disturbing the pellet. Air-dry the pellet.
11. For mass spectrometric analysis resuspend the pellet in appropriate solution compatible with MALDI-MS (protein characteristic is important for determination the appropriate solution) followed by essential dilution step according to the protocols compatible with MALDI-MS. Use at least 100 µl to perform resuspension.

Protein precipitation protocols used after isolation from the DiaEasy Dialyzer Tubes

Trichloroacetic acid (TCA) precipitation procedure (for protein)

1. Add equal volume of 20% TCA to the tube containing the extracted protein solution and mix properly. For example, add 3 ml of 20% TCA to a 3 ml sample.
2. Incubate 60 min in 4°C.
3. Centrifuge at 4°C for 30 min at 20,000 g and carefully discard the supernatant.
4. Add 2 ml cold acetone and incubate at -20°C for 60 min.
5. To increase protein precipitation yield, incubate the samples overnight at -20°C.
6. Centrifuge the sample at 4°C for 30 min at 20,000 g.
7. Discard supernatant and air-dry the pellet.
8. Resuspend the pellet in 0.1 M NaOH (use at least 0.1 ml to perform resuspension).

MS precipitation procedure (recommended when protein-bound SDS need to be removed)

1. Add 1:10 by volume of your choice of MS buffer to the protein containing solution and mix properly. For example, add 0.3 ml of MS buffer to a 3 ml sample.
2. Incubate for 15 min at room temperature.
3. Add 1: 2 by volume of 20% TCA and mix properly. For example, add 1.65 ml of 20% TCA to a 3.3 ml sample.
4. Incubate for 1 hour at 4°C.
5. Centrifuge the sample at 4°C for 30 min at 20,000 g and carefully decant the supernatant without disturbing the pellet.
6. Add 2 mL of ice-cold acetone.
7. Incubate at -20°C for 30 min and centrifuge the sample at 4°C for 30 min at 20,000 g.
8. To increase protein precipitation yield, incubate the samples overnight at -20°C.
9. Carefully decant the supernatant without disturbing the pellet. Air-dry the pellet.
10. Resuspend the pellet in a suitable buffer solution or 0.1 M NaOH (use at least 0.1 ml to perform resuspension).

Technical Support

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