ab286927 – DiaEasy Dialyzer (800 µl) MWCO 3.5 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/ab286927

Storage and Stability

Store all components of the kit at room temperature (RT).

Materials Supplied

Item	Quantity (10 Vial)	Quantity (25 Vial)	Quantity (100 Vial)	Storage Condition
DiaEasy Dialyzer Tubes	10	25	100	RT
DiaEasy Floating Rack	2	5	10	RT
DiaEasy Supporting Tray	2	5	10	RT

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 800 µl of dH2O. Incubate for at least 5 min. Empty the tube.

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

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

Δ Note: *Sample volume should be in the range of 50-800 µl. If a 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 a large volume (usually 100 to 1000-fold that of the sample) of the desired buffer. The floating rack can hold 1-4 DiaEasy Dialyzer Tubes. Adjust the stir bar speed.
4. 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: *User must determine exact equilibration times for the dialysis.*

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

Δ Note: *If the 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 the samples in the DiaEasy Dialyzer tubes, or use already dialyzed samples, and place them on the microtube rack stand.
2. Let the samples evaporate on the bench top (using a fan to increase airflow across the membrane to speed up the evaporation process), checking every 10 min or less to prevent full evaporation and drying of samples.

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 800 µl of dH2O. Incubate for at least 5 min. Empty the tube.

Δ Note: *Check carefully that no dH2O is leaking from the tube. Water absorption, by the dry membrane, causes a decrease of the 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 1 cm x 0.5 cm.
3. Transfer the gel slice to a DiaEasy Dialyzer tube. Fill the tube with 0.7 – 0.8 ml dH2O. Close the tube gently. 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 hold 1-4 DiaEasy Dialyzer tubes.

Δ Note: *The arrow on the cap should be pointing upwards. The two membranes of the DiaEasy Dialyzer tube must be 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 an electric current (usually at 80-150 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 needs 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 1.5 ml microcentrifuge tube. Pipette on the inner side of the membrane.
10. Centrifuge the microcentrifuge tube for 1 min at maximum speed. This step will remove gel residues.

11. Transfer the nucleic acid-containing solution to a clean 1.5 ml microcentrifuge tube.

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

Elution Time Tables: 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 100-150 volt.

DNA		RNA	
Fragment size (bp)	**Elution time (min)**	**Fragment size (nt)**	**Elution time (min)**
100	10-20	100	15-25
300	15-25	400	25-35
500	20-30	600	35-45
822	25-35	1000	45-55
1044	30-40		
2700	45-55		

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

Fragment size (bp)	Elution time (min)
100-200	10-20
500-700	15-20
1000	20-30
4361	25-35
6557	45-55
9416	55-65
23130	70-80

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, for a 700 µl sample, add 70 µl of 3 M Potassium acetate, pH 5.2, and 500-700 µl isopropanol.
2. Mix gently by inverting the tube several times.

Δ Note: *Addition of carrier (e.g. 20 µg tRNA or 20 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 x 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 x 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 difficult to redissolve, especially if it is of high molecular weight.*

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 electroelution (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 0.8 ml of dH2O. Incubate for at least 5 min. Empty the tube.

Δ Note: *Check carefully that no dH2O is leaking from the tube. Water absorption, by the dry membrane, causes a decrease of the 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 1 cm x 0.5 cm.
4. Transfer the gel slice to a DiaEasy Dialyzer tube. Fill the tube with protein-running buffer (0.7-0.8 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 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: Fully immerse the DiaEasy Dialyzer tubes with the tray in the buffer.

8. Pass an electric current (usually at 100 volts) until the protein exits from the gel slice.
9. Electroelution time needs to be adjusted for each individual sample. It takes at least 85 min for BSA protein to be electroeluted 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 1.5 ml microcentrifuge tube. Pipette on the inner side of the membrane.

12. Centrifuge the microcentrifuge tube for 1 min at maximum speed. This step will remove gel residues.
13. Transfer the protein-containing solution to a clean 1.5-ml microcentrifuge tube.

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 the extracted protein directly with a clean DiaEasy Dialyzer tube.*

Elution Time Table: 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. Electroelution time at the elution step should 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

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 0.8 ml of dH2O. Incubate for at least 5 min. Empty the tube.

Δ Note: *Check carefully that no dH2O is leaking from the tube. Water absorption, by the dry membrane, causes a decrease of 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 1 cm x 0.5 cm.*

3. Transfer the gel slice to a DiaEasy Dialyzer Tube. 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 trapping 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: *Fully immerse the DiaEasy Dialyzer Tubes with the tray in the buffer.*

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

Δ Note: *The electro-elution time should be adjusted for each individual sample. It takes at least 2 hours for BSA protein to be electroeluted from a 10% SDS-PAGE gel slice.*

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 1.5 ml microcentrifuge tube.

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

9. Centrifuge the microcentrifuge tube for 1 min at maximum speed. This step will remove gel residues.
10. Transfer the protein-containing solution to a clean 1.5 ml microcentrifuge tube.

Protein Precipitation for Analysis by MALDI-MS Protocol

1. Add 1:10 by volume of MS buffer of your choice into the protein-containing solution and mix properly. For example, add 70 µl of MS buffer to a 700 µl sample.
2. Incubate for 15 min at room temperature.
3. Add 1:5 by volume of 50% Trichloroacetic acid (TCA) and mix properly. For example, add 154 µl of 50% TCA to a 770 µl sample and buffer mixture.
4. Incubate for 1 hour at 4°C.
5. Centrifuge the sample at 4°C for 30 min at 20,000 x 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, then centrifuge the sample at 4°C for 30 min at 20,000 x g.
9. To increase protein precipitation yield, incubate the samples over night 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 steps according to the protocols compatible with MALDI-MS. Use at least 20 µl to perform resuspension.

Protein precipitation protocols used after isolation from the DiaEasy Dialyzer Tubes

Trichloroacetic acid (TCA) precipitation procedure (for protein)

1. Add an equal volume of 20% TCA to the microcentrifuge tube containing the extracted protein solution and mix properly. For example, add 700 µl of 20% TCA to a 700 µl sample.
2. Incubate for 60 min in 4°C.
3. Centrifuge at 4°C for 30 min at 20,000 x g and carefully discard the supernatant.
4. Add 500 µl cold acetone and incubate at -20°C for 60 min.
5. Centrifuge the sample at 4°C for 30 min at 20,000 x g.
6. To increase protein precipitation yield incubate the samples overnight at –20°C.
7. Discard supernatant and air-dry the pellet.
8. Resuspend the pellet in 0.1 M NaOH or dH2O (use at least 20 µl to perform resuspension). If dH2O is used for resuspension, incubate the sample for 5 min in 60°C, resuspend the sample and incubate for 5 min more at 60°C.

MS precipitation procedure (recommended when SDS bound to proteins needs 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 70 µl of MS buffer to a 700 µl sample.
2. Incubate for 15 min at room temperature.
3. Add 1: 2 by volume of 20% TCA and mix properly. For example, add 385 µl of 20% TCA to a 770 µl sample.
4. Incubate for 1 hour at 4°C.
5. Centrifuge the sample at 4°C for 30 min at 20,000 x g and carefully decant the supernatant without disturbing the pellet.
6. Add 500 µl 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 x 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 20 µl to perform resuspension).

Technical Support

For all technical or commercial enquiries please go to:
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