D-Tube™ Dialyzers: Mini, Midi, Maxi, and D-Tube96™

Table of Contents

USA and Canada
Tel (800) 628-8470
biosciencehelp@
emdchemicals.com

Europe

France	**Germany**	**Ireland**	**United Kingdom**	**All other European Countries**
Freephone	Freecall	Toll Free	Freephone	+44 115 943 0840
0800 126 461	0800 100 3496	1800 409 445	0800 622 935	

All Other Countries
Contact Your Local Distributor
www.merck4biosciences.com
biosciencehelp@
emdchemicals.com

techservice@merckbio.eu

www.merck4biosciences.com

About the Kits

D-Tube™ Dialyzer Mini, MWCO 6–8 kDa	10 tubes	71504-3
D-Tube Dialyzer Mini, MWCO 12–14 kDa	10 tubes	71505-3
D-Tube Dialyzer Midi, MWCO 3.5 kDa	10 tubes	71506-3
D-Tube Dialyzer Midi, MWCO 6–8 kDa	10 tubes	71507-3
D-Tube Dialyzer Maxi, MWCO 3.5 kDa	10 tubes	71508-3
D-Tube Dialyzer Maxi, MWCO 6–8 kDa	10 tubes	71509-3
D-Tube Dialyzer Maxi, MWCO 12–14 kDa	10 tubes	71510-3
D-Tube96™ Dialyzer, 6-8 kDa	1 kit	71712-3
D-Tube96 Dialyzer, 12-14 kDa	1 kit	71713-3
D-Tube Electroelution Accessory Kit	1 kit	71511-3

Description

D-Tube™ Dialyzers* provide an extremely convenient and versatile system for the manipulation of biological samples. Two modes of operation, dialysis and electroelution, enable efficient buffer exchange or gel extraction of protein, DNA, or RNA, in a single-tube format over a wide range of conditions. There is no need for a syringe, microcentrifuge, dedicated electroelution apparatus, or laborious steps during sample manipulations. The samples are added and removed using a standard laboratory pipet. D-Tube Dialyzers are also ideally suited for sample concentration by evaporation because of their dual membranes (which enable easy monitoring of evaporation progress) and large surface area.

D-Tube Dialyzers are available in several volume capacities: Mini (10–250 µl), Midi (50–800 µl), and Maxi (100–3000 µl). The D-Tube Dialyzer Maxi kits are provided with two caps to allow easy adjustment of capacity from 100–2000 µl to 2000–3000 µl. The D-Tube96™ device includes all features of the D-Tube Dialyzer Mini in convenient, 96-tube configuration. All D-Tube Dialyzer membranes consist of ultra-clean, EDTA-treated regenerated cellulose. Membranes are free of sulfur and heavy metals. Nonspecific protein binding by the membrane is negligible, as tested using proteins of acidic, neutral, or basic pI in concentrations ranging from 35–70 µg/ml.

In addition to their utility for dialysis, D-Tube Dialyzers are unique tools for extraction and electroelution of any protein, protein-protein, and protein-nucleic acid complexes from non-denaturing and denaturing (SDS) polyacrylamide gels in less than two hours. The procedure efficiently recovers proteins (see Table 1 below) and simultaneously removes ampholytes from protein samples separated on 2D gels. Extracted proteins are compatible with applications such as MALDI-MS, immunization for antibody production (provided that running buffer is removed by dialysis), HPLC analysis, or peptide mapping. In addition, D-Tube Dialyzers can be used for oligonucleotide, RNA, and DNA extraction from polyacrylamide and agarose gels. Efficient extraction occurs for nucleic acids ranging in size from 15-nt oligonucleotides to 80 kbp double-stranded DNA. No specialized electroelution apparatus is required, as the D-Tube Electroelution Accessory Kit provides supporting trays fitting all three D-Tube Dialyzers (Mini, Midi, and Maxi) and that are compatible with most commercially-available horizontal electrophoresis units. Reagents are also provided for protein and nucleic acid precipitation following electroelution. In addition, the kit contains buffer optimized for thorough removal of SDS from the extracted protein samples, rendering them compatible with MALDI-MS analysis.

*U.S. Patent No. 7,074,313

Table 1. Typical recoveries following electroelution	
DNA or RNA from agarose gel	>90%
DNA or RNA from polyacrylamide gel	>90%
Protein from SDS-PAGE	>60%

Components

D-Tube™ Dialyzer Kits

- 10 D-Tube™ Dialyzers (Mini, Midi, or Maxi)
- 1 Floating Rack (Mini, Midi, or Maxi)

D-Tube96™ Dialyzer Kits

- 1 D-Tube96™ Dialyzer (6-8 kDa or 12-14 kDa)
- 96 D-Tube96 Dialyzer caps
- 1 Aluminum Plate Sealer

D-Tube Electroelution Accessory Kit

- 1 Supporting Tray Mini
- 1 Supporting Tray Midi
- 1 Supporting Tray Maxi
- 1 ml MS Precipitation Buffer
- 10 ml TCA, 20%
- 2 × 1 ml 3 M Sodium Acetate, pH 5.2

Storage

Store all components at room temperature.

Dialysis and Sample Concentration

Use the following protocol for dialysis and/or sample concentration using the D-Tube™ Dialyzers Mini, Midi, or Maxi.

The D-Tube Dialyzer Maxi kits are provided with two caps. For sample volumes less than 2 ml, use the 2-ml cap. For sample volumes between 2 and 3 ml, use the 3-ml cap. See Figure 1 below.

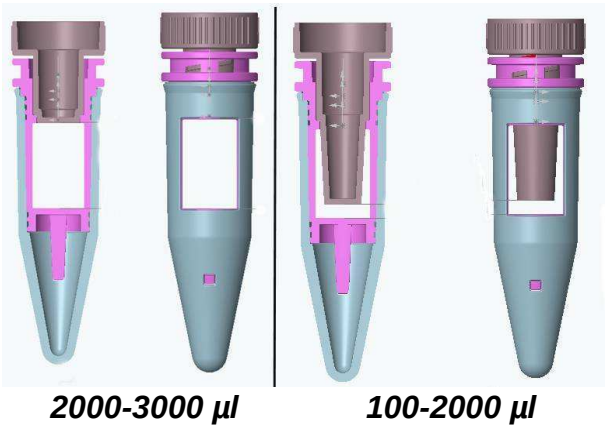

Figure 1. D-Tube Dialyzer Maxi kits are provided with a 3-ml cap (left panel) and a 2-ml cap (right panel). Cross-sections of the device and cap assembly are shown to the left of each panel. Side views of the assembled device are shown to the right of each panel.

1. Remove cap from D-Tube™ Dialyzer and add amount of deionized water indicated in Table 2. Incubate upright for at least 5 min. Screw cap on gently.

Table 2

D-Tube™ Dialyzer	Volume of deionized water	Sample volumes
Mini	250 µl	10–250 µl
Midi	800 µl	50–800 µl
Maxi (2-ml cap)	2–3 ml	100–2000 µl
Maxi (3-ml cap)	2–3 ml	2–3 ml

Note: It is critical to pre-hydrate membranes prior to adding sample. Do not skip Step 1 above. The water level will decrease as the dry membranes absorb some of the water. Do not touch the surface of the membrane or squeeze the membrane, as this can result in breakage. Carefully check that there is no water leaking from the tube. We do not recommend re-using membranes that have been allowed to dry.

2. Remove cap. Remove water from tube using a pipet. Add sample and screw cap on gently. Do not fill device to capacity if dialyzing a sample of high osmolarity against a buffer of low osmolarity. Under this condition, significant sample volume increase will occur during dialysis. If inadequate space is available for volume expansion, membranes can burst.

Tip: If loading a small sample volume (Midi ≤100 µl, Mini ≤10 µl), load sample near inner membrane of D-Tube Dialyzer, taking care not to puncture the membrane.

3. Place D-Tube Dialyzer in Floating Rack provided. Place rack in beaker containing 100- to 1000-fold sample volume of desired dialysis buffer and a stir bar. The entire surface of the membranes must be submerged in buffer.
4. Stir gently but thoroughly for at least 3 h.

Note: Optimum equilibration times for dialysis are specific to each application and must be determined by the user. Low molecular weight salts and buffers (e.g., Tris-HCl or potassium phosphate) generally equilibrate within 3 h. To ensure that samples reach equilibrium, dialysis may be conducted overnight. Equilibration times for viscous samples will be longer.

5. Change dialysis buffer as needed. The number of buffer changes required will depend upon the specific application.
6. **Optional:** If sample volume increased during dialysis, place D-Tube Dialyzer in microtube rack. Let sample evaporate on bench top. Increasing airflow across membrane will speed up the process. Check volume at least every 10 min to prevent excess evaporation.

Note: Because no diffusion occurs during evaporation, small molecules in the sample (buffers, salts, reducing agents, etc.) will also be concentrated.

7. Remove cap from D-Tube Dialyzer. Carefully remove sample from D-Tube Dialyzer with a pipet. Transfer sample to clean microcentrifuge tube.

Dialysis Using D-Tube96™ Dialyzers

D-Tube96™ Dialyzers can be used for high-throughput dialysis of proteins, oligonucleotides, RNA and DNA. D-Tube96 Dialyzers are ideally suited for buffer exchange of protein samples into an assay buffer after determining optimal refolding conditions with the iFOLD™ Protein Refolding Systems (see User Protocols TB457 and TB467).

The D-Tube96 Dialyzer device is modular. If less than 96 tubes are required, excess tubes can be removed and saved for use at a later time. Caps are provided in the kit.

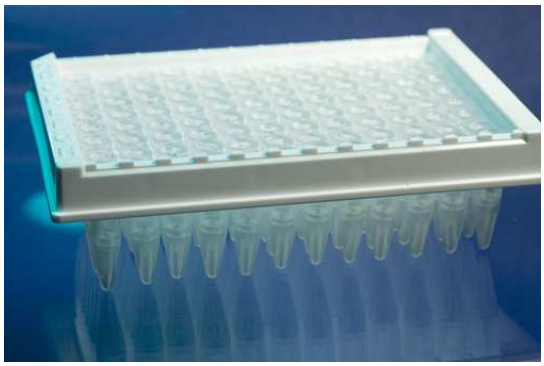

Figure 2. D-Tube96™ Dialyzer

Notes: *Ensure that individual D-Tube96™ tubes are held securely in the 96-plate frame. The top of the D-Tube96 Dialyzer unit should be 1-2 mm above the white plastic grid.*

The membranes of the individual D-Tube96 tubes do not have to be in a particular orientation. Buffer exchange will occur at approximately the same rate for samples, regardless of membrane orientations.

1. Remove any D-Tubes that will not be used.
2. Add 250 µL of deionized water to each of the remaining D-Tubes. To prevent formation of bubbles, touch the pipette tips to the bottom of D-Tubes and slowly withdraw the tips as water is expelled. Incubate for at least 5 min.

Note: It is critical to pre-hydrate membranes prior to adding sample. Do not skip Step 2 above. The water level will decrease as dry membranes absorb some of the water. Do not touch the surface of the membranes or squeeze the membranes, as this can result in breakage. Carefully check that there is no water leaking from the tubes. We do not recommend re-using membranes that have been allowed to dry.

3. Remove water from all tubes, taking care not to puncture the membranes.
4. Fill individual D-Tube96 tubes with samples. To prevent formation of bubbles, touch the pipette tips to the bottom of D-Tubes and slowly withdraw the tips as water is expelled.

Note: *Individual tube capacity is 250 µl. However, depending on the composition of samples and dialysis buffer, sample volume may increase or decrease significantly.*

If using D-Tube96 Dialyzers to exchange buffer following use of the iFOLD™ Protein Refolding System, add 125 µl of individual refolding reactions to D-Tube96 tubes. Several of the refolding buffers in the iFOLD matrix contain high salt concentrations (>0.5 M), resulting in significant sample volume increase when dialyzing against typical assay buffers. If inadequate space is available for volume expansion, membranes can burst.

5. Seal the D-Tube96 Dialyzers with the adhesive aluminum plate sealer. Ensure that rim of each D-Tube96 tube is in contact with foil.
6. Float the D-Tube96 device in beaker containing dialysis buffer and a stir bar. Use a dialysis vessel large enough to allow the D-Tube96 device to spin freely. If the device is held static, the efficiency of dialysis may be reduced.
7. Stir gently but thoroughly for at least 4 hours.
8. Remove aluminum plate sealer. Carefully remove samples with a pipet and transfer to clean microcentrifuge tubes.
9. **Optional:** If sample volumes increased during dialysis, place D-Tube96 Dialyzer in microtube rack. Let samples evaporate on bench top. Increasing airflow across membrane will speed up the process. Check volume at least every 10 min to prevent excess evaporation.

Note: *Because no diffusion occurs during evaporation, small molecules (buffers, salts, reducing agents, etc.) will also be concentrated.*

Protein Extraction from Polyacrylamide Gels

The following protocols describe the use of D-Tube™ Dialyzer devices for electroelution of proteins or protein complexes from a variety of gels. Electroelution can be performed with non-denaturing and denaturing (SDS) polyacrylamide gels, one-dimensional and two-dimensional polyacrylamide gels, and isoelectric focusing gels.

For optimal electroelution of protein from polyacrylamide gels, avoid fixation of proteins before electroelution (e.g., treatment of the gel with methanol, acetic acid, etc.). **Fixation will greatly reduce extraction yield**. RAPIDStain™ Reagent (Calbiochem Cat. No. 553215) is an ultrasensitive Coomassie-based reagent for staining polyacrylamide gels and is compatible with extraction. RAPIDStain does not require mixing or preparation, fixation, or destaining, and stains protein within 5-10 min.

D-Tube Dialyzers can be used to extract proteins for analysis by MALDI-MS (Matrix-assisted Laser Desorption/Ionization Mass Spectrometry). The D-Tube Dialyzers enable high protein recovery, and the MS Precipitation Buffer in the D-Tube Electroelution Accessory Kit thoroughly removes SDS. This can facilitate more sensitive MALDI-MS analysis. If analysis by MALDI-MS will be performed, follow the electroelution protocol below with the ***Notes for MALDI-MS***.

Electroelution Protocol - Proteins

1. Remove cap from D-Tube Dialyzer. Add deionized water; see Table 3 for recommended volumes. Incubate upright for at least 5 min. Screw cap on gently.

Note: *It is critical to pre-hydrate membranes prior to adding sample. Do not skip Step 1 above. The water level will decrease as dry membranes absorb some of the water. Do not touch the surface of the membranes or squeeze the membranes, as this can result in breakage. Carefully check that there is no water leaking from the tube. We do not recommend re-using membranes that have been allowed to dry.*

2. After staining gel, excise gel slice containing desired protein band or spot using a clean, sharp scalpel. Trim away excess gel. (See information above regarding protein staining procedures. Do not use fixed gels.)
3. Remove cap from D-tube Dialyzer. Remove water from tube using a pipet, taking care not to puncture the membranes. Transfer gel slice to D-Tube Dialyzer. The maximum gel slice capacity per device is indicated in Table 3. Fill tube with protein running buffer to top of membranes (see Table 3 for recommended volumes). Typical SDS-PAGE running buffers may be used for standard MS applications; see below for running buffer composition when samples are to be analyzed using MALDI-MS. Avoid introducing air bubbles in tube, as they will interfere with electroelution. Screw cap on gently.

Table 3.

D-Tube™ Dialyzer	Deionized water added to tube	Maximum size gel slice	Running buffer added to tube
Mini	0.25 ml	0.4 cm x 1.1 cm x 1 mm	0.25 ml
Midi	0.8 ml	0.5 cm x 1 cm x 1 mm	0.7–0.8 ml
Maxi	2.5–3.0 ml	2 cm x 1 cm x 1 mm	2.5–3.0 ml

Note: *Separate large gel slices into multiple tubes.*

Note for MALDI-MS: *A standard protein running buffer for MALDI-MS is 250 mM Tricine, 25 mM Tris-base, 0.025% SDS, pH 8.5*

4. Place tube in the appropriate size Supporting Tray (See Figure 3 below). **The two membranes of each D-Tube Dialyzer must be perpendicular to the electric field to permit electric current to pass through the tube.**

Note: *The supporting trays can hold 1–4 Mini or Midi D-Tubes, or 1–3 Maxi D-Tubes.*

Figure 3. Insertion of D-Tube™ Dialyzer in Supporting Tray Midi showing correct orientation of membranes, which must be perpendicular to the electric field.

5. Place Supporting Tray containing D-Tube™ Dialyzer(s) in a horizontal electrophoresis tank (such as tanks typically used for nucleic acid agarose electrophoresis) containing protein running buffer (see Figure 4).

Important: *Fully immerse D-Tube Dialyzer(s) in protein running buffer.*

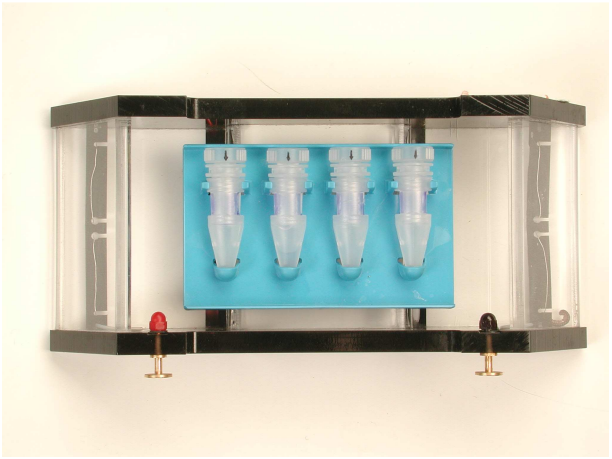

Figure 4. Supporting Tray containing four D-Tube Dialyzers in a horizontal electrophoresis tank. Membranes of each tube are perpendicular to the electric field.

6. Apply electric current (usually 100 V) until the protein exits the gel slice. For general electroelution times, see Table 4 (page 8). The optimum electroelution time will need to be determined for each sample and gel concentration. The minimum electroelution time for BSA (66 kDa) from a 10% SDS-PAGE gel is at least 85 min.

Tip: *Elution of protein from gel can be monitored by referring to the protein stain. Once most of the stain has exited the gel slice, the majority of protein is likely eluted.*

Note for MALDI-MS: *Due to the lower amount of SDS generally used for samples that will be analyzed by MALDI-MS, the usual electric current for elution is 150 V. The minimum electroelution time for BSA from a 10% SDS-PAGE under these conditions is at least 2 h.*

7. Release protein from membrane by reversing polarity of electric current for 2 min.

8. Gently open D-Tube™ Dialyzer. Pipet eluate up and down at least 5 times on inner side of membrane, using care to avoid gel slice and not puncture the membrane. Transfer eluate to clean microcentrifuge tube.
9. Centrifuge eluate for 1 min at 14,000 × *g* to pellet gel residues.
10. Transfer supernatant to clean microcentrifuge tube. The protein can be used immediately, concentrated by standard concentration methods, precipitated (see *Protein Precipitation* on p 9), or dialyzed. If desired, the same D-Tube Dialyzer used for electroelution may be used for dialysis. Carefully remove gel slice, taking care not to puncture the membranes. See *Dialysis and Sample Concentration* on p 3.

Elution Time - Proteins

Elution time is affected by the size of the protein to be eluted, the applied voltage, the size of the gel slice, the ratio of polyacrylamide:bisacrylamide, and the concentration of polyacrylamide. Table 4 indicates minimum times needed to extract protein from a 10% SDS-polyacrylamide gel (29:1 polyacrylamide:bisacrylamide) at 100 V.

Important: The optimal electroelution time may vary depending on the individual sample.

Table 4. Electroelution times

Protein (kDa)	Time (min)		
	Mini	Midi	Maxi
14	30–40	35–45	50–60
18.4	35–45	*	55–65
19–26	*	45–55	*
25	40–50	*	70–80
29	*	55–65	*
35	50–60	*	110–120
40	*	60–70	*
45	55–65	65–75	130–140
50	*	75–85	*
66	70–80	85–95	150–160
81	*	105–115	*
116	90–100	120–130	180–190
128	*	140–150	*

* Not determined

Protein Precipitation

Alternatively, the ProteoExtract™ Protein Precipitation Kit (Calbiochem Cat. No. 539180) is available for fast and efficient precipitation and clean-up of protein samples.

Dialyzed protein samples can be precipitated according to standard methods using reagents provided in the D-Tube™ Electroelution Accessory Kit. Brief precipitation methods are described below.

Trichloroacetic acid (TCA) precipitation

1. Add 1 volume TCA, 20% to sample. Mix thoroughly by vortexing.
2. **Optional:** For samples to be analyzed by either standard MS or MALDI-MS, begin by adding 0.l vol MS Precipitation Buffer. Mix thoroughly, incubate for 15 min at room temperature, and then add 0.5 vol 20% TCA (for standard MS) or 0.2 vol 50% TCA (for MALDI-MS; 50% TCA is not provided in the kit). Mix thoroughly by vortexing and proceed to Step 2.
3. Incubate 1 h at 4°C.
4. Centrifuge sample at 4°C for 30 min at 14,000 × *g*. Decant supernatant, taking care not to disturb pellet.
5. Add ice-cold 100% acetone (not provided in the kit) to wash the pellet (e.g., use 500 µl–2 ml acetone depending on pellet size). Mix thoroughly by vortexing.
6. Incubate at –20°C for at least 30 min.

Tip: *Incubating samples at –20°C overnight can incr ease protein precipitation efficiency.*

7. Centrifuge at 4°C for 30 min at 14,000 × *g*. Decant supernatant, taking care not to disturb pellet.
8. Let pellet dry thoroughly (about 1 h) in a hood. Resuspend pellet in an appropriate volume 0.1 M NaOH (not provided in the kit) or deionized water.

Note: *It is important that pellet dry completely; otherwise, it will be very difficult to resuspend. If using deionized water to resuspend pellet, first incubate pellet for 5 min at 60°C. Add deionized water. Incubate again for 5 min at 60°C.*

Note for MALDI-MS: *Resuspend pellet in an appropriate amount of solution suitable for MALDI-MS. The characteristics of the protein are important for determining the appropriate solution.*

Nucleic Acid Extraction From Gels

Use the following protocol for extracting DNA or RNA from polyacrylamide or agarose gels.

1. Remove cap from D-Tube Dialyzer. Add appropriate volume of deionized water (see Table 9 on p 9 for recommended volumes). Incubate upright for at least 5 min. Screw cap on gently.

Note: *It is critical to pre-hydrate membranes prior to adding sample. Do not skip Step 1 above. The water level will decrease as dry membrane absorbs some of the water. Do not touch the surface of the membranes or squeeze the membranes, as this can result in breakage. Carefully check that there is no water leaking from the tube.*

Never re-use membranes that have been allowed to dry.

2. Excise gel slice containing desired DNA or RNA fragment using a clean, sharp scalpel. Trim away excess gel.
3. Remove cap. Remove water from tube using a pipet, taking care not to puncture the membrane. Transfer gel slice to D-Tube Dialyzer. The maximum gel slice capacity per device is indicated in Table 9. Fill tube with running buffer to top of the membranes (see Table 9 for recommended volumes). Avoid introducing air bubbles in the tube, as they will interfere with electroelution. Screw cap on gently.

Table 9.

D-Tube™ Dialyzer	Deionized water added to tube	Maximum size gel slice	Running buffer added to tube
Mini	0.25 ml	0.4 cm × 1.1 cm	0.25 ml
Midi	0.8 ml	0.5 cm × 1 cm	0.7–0.8 ml
Maxi	2.5–3.0 ml	2 cm × 1 cm	2.5–3.0 ml

Note: *For electroelution, use the same running buffer that was used in the gel separation step. Separate larger gel slices into multiple tubes.*

4. Place tube in the Supporting Tray (See Figure 3 on p 7). **The two membranes of D-Tube™ Dialyzer must be positioned perpendicular to the electric field to permit electric current to pass through tube.**
5. Place Supporting Tray containing D-Tube Dialyzer(s) in a horizontal electrophoresis tank containing running buffer (see Figure 4 on p 7).
6. Apply electric current (usually 80–150 volts) until nucleic acid exits gel slice. The optimum electroelution time must be determined for each sample and gel concentration. For general electroelution times see Tables 10–12.

Tip: *If gel is stained with ethidium bromide or SYBR® Green, elution of nucleic acid from gel can be monitored with a hand-held or table UV lamp.*

7. Release nucleic acid from membrane by reversing polarity of electric current for 2 min.
8. Gently open D-Tube Dialyzer. Pipet eluate up and down at least 5 times on inner side of membrane, taking care to avoid gel slice and to not puncture the membrane. Transfer eluate to clean microcentrifuge tube.
9. Centrifuge eluate for 1 min at 14,000 × *g* to pellet gel residues.
10. Transfer supernatant to clean microcentrifuge tube. Concentrate nucleic acids using standard precipitation protocols (see section entitled *Nucleic Acid Precipitation* below).

Elution Time – Nucleic Acids

Elution time is affected by size of the nucleic acid fragment, the applied voltage, the concentration of the gel, the size of the gel slice, and the ratio of polyacrylamide:bisacrylamide. Tables 10-12 indicate minimum times needed to extract DNA and RNA from polyacrylamide and agarose gels.

Important: *The optimal electroelution time may vary depending on the individual sample.*

Tables 10 & 11. Minimum times required to elute various DNA or RNA fragments from a native or denaturing 4% polyacrylamide gel (29:1 polyacrylamide:bisacrylamide) at 100–150 volts.

Table 10. Electroelution times

DNA	Time (min)		
Fragment size (bp)	Mini	Midi	Maxi
100	10–15	10–20	10–15
200	15–20	*	15–20
300	*	15–25	*
500	30–35	20–30	30–35
822	*	25–35	*
1000	55–60	*	55–60
1044	*	30–40	*
1400	75–80	*	75–80
2700	*	45–55	*

*Not determined

Table 11. Electroelution times

RNA	Time (min)
Fragment size (nt)	Midi
100	15–25
400	25–35
600	35–45
1000	45–55

Table 12. Minimum times required to elute DNA fragments from a 1% agarose gel at 80–110 volts.

Table 12. Electroelution times

DNA	Time (min)		
Fragment size (bp)	Mini	Midi	Maxi
100–200	*	10–20	*
500–700	10–15	15–20	10–15
1000	15–20	20–30	15–20
2000	25–30	*	25–30
4361	*	25–35	*
5000	40–45	*	40–45
6557	*	45–55	*
8000	50–55	*	50–55
9416	*	55–65	*
1000	55–60	*	55–60
23130	*	70–80	*

*Not determined

Nucleic Acid Precipitation

Dialyzed nucleic acid samples can be precipitated according to standard methods using 3 M Sodium Acetate, pH 5.2 provided in the D-Tube™ Electroelution Accessory Kit. A brief precipitation protocol is described below.

1. Add 0.1 vol 3 M Sodium Acetate, pH 5.2 and 2 vol 100% ethanol or 1 vol 100% isopropanol to nucleic acid sample. Vortex briefly.

Note: The addition of Pellet Paint® Co-Precipitant (Cat. No. 69049) or Pellet Paint NF Co-Precipitant (Cat. No. 70748) to nucleic acid sample facilitates recovery during precipitation. Pellet Paint NF is recommended for applications involving Applied Biosystems automated sequencers.

2. Incubate at room temperature for 5 min.
3. Centrifuge at 14,000 × *g* for 5 min, remove supernatant, and rinse pellet with 70% ethanol. Vortex briefly and repeat ethanol wash.
4. Remove supernatant. Wash pellet with several volumes 100% ethanol and centrifuge as above. After removing final supernatant, dry pellet completely to remove residual ethanol. Resuspend pellet in deionized water or buffer of choice.

Troubleshooting - Electroelution

Symptom	Possible cause	Solution
Low yield	Insufficient elution time	Increase elution time. Increase applied voltage.
	Polarity of current was not reversed after elution	Reverse polarity of current for 2 min at end of elution procedure.
	Tube not fully immersed in buffer of electrophoresis tank	Fully immerse tube in buffer of electrophoresis tank, using supporting tray.
	Gel slice not fully immersed in buffer inside tube, or air bubbles are present	After inserting gel slice in tube, add running buffer or deionized water to top of the two membranes. Make sure no air bubbles are present in tube.
	More than recommended gel volume inserted in tube	Do not cut large gel slices or place multiple pieces in the same tube. Separate large gel slices into multiple tubes.
	Tube oriented incorrectly, electric current does not pass through tube	The two membranes of the D-Tube™ Dialyzer must be perpendicular to electric field.
Long elution times	Low applied voltage	Increase applied voltage.
	Gel slice is not fully immersed in buffer inside tube, or air bubbles are present	After inserting gel slice in tube, add running buffer or deionized water to top of the two membranes. Make sure no air bubbles are present in tube.
	Tube not fully immersed in buffer of electrophoresis tank	Fully immerse tube in buffer of electrophoresis tank, using supporting tray.
Volume of solution reduced after elution	Membrane was dry when sample was added	Wet membrane for 5 min with deionized water before adding sample.
	Pinhole in membrane	Use new D-Tube Dialyzer.

Related Products

	MWCO	Volume	Kit Size	Catalog Number
D-Tube™ Dialyzer	3.5 kDa (blue)	3–10 ml	10 tubes	71739-3
D-Tube Dialyzer Mega	3.5 kDa (blue)	3–10 ml	50 tubes	71739-4
D-Tube Dialyzer Mega	6–8 kDa (pink)	3–10 ml	10 tubes	71740-3
D-Tube Dialyzer Mega	6–8 kDa (pink)	3–10 ml	50 tubes	71740-4
D-Tube Dialyzer Mega	3.5 kDa (blue)	10–15 ml	10 tubes	71742-3
D-Tube Dialyzer Mega	3.5 kDa (blue)	10–15 ml	50 tubes	71742-4
D-Tube Dialyzer Mega	6–8 kDa (pink)	10–15 ml	10 tubes	71743-3
D-Tube Dialyzer Mega	6–8 kDa (pink)	10–15 ml	50 tubes	71743-4
D-Tube Dialyzer Mega	3.5 kDa (blue)	15–20 ml	10 tubes	71745-3
D-Tube Dialyzer Mega	3.5 kDa (blue)	15–20 ml	50 tubes	71745-4
D-Tube Dialyzer Mega	6–8 kDa (pink)	15–20 ml	10 tubes	71746-3
D-Tube Dialyzer Mega	6–8 kDa (pink)	15–20 ml	50 tubes	71746-4
Floating Racks, Mega	–	–	10 racks	71748-3