FLEXTUBE HANDBOOK

MIDI

IBI FLEXTUBES ARE MANUFACTURED BY GENE-BIO-APPLICATION LTD. - ISRAEL

IBI/GEBA FLEXTUBES ARE COVERED BY PATENT APPLICATION WO0190731

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A. Packaging and Handling

- Wearing gloves is highly recommended when handling the kit contents.
- IBI FlexTubes are autoclaved and bacterial free.
- IBI FlexTube membranes are ultra-clean, sulfur and heavy metal free, and EDTA treated.

B. Applications

- Extraction of proteins, RNA, DNA or oligonucleotides (>20 nt) from polyacrylamide, agarose or any gel matrix in any running buffer.
- Extraction of protein-protein, DNA-protein or RNA-protein complexes.
- Dialysis or buffer exchange of small volumes (50-800µl).
- Preparation of protein samples for MALD-MS.
- Samples concentration

C. Kit Contents

♦ IBI FlexTubes	2 / 10 / 30 / 50 / 100 pieces
♦ Supporting tray (for electro elution protocol)	1ea. (select kits)
♦ Floating rack (for dialysis protocol)	1ea. (select kits)
♦ Information and Protocol Manual	1ea.

D. Storage Conditions

IBI FlexTube kits should be stored in a dry place at room temperature (15-25°C). Under these conditions, IBI FlexTube kits can be stored for up to 12 months without any deterioration in performance or quality. For longer storage times, it is recommended that IBI FlexTube kits be stored in a cool dry place, like a refrigerator.

E. Product Limitations

IBI FlexTube kits are developed, designed, and sold for research purposes only. They are not to be used for human diagnostic purposes or drug production, nor for producing any substance intended to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration (USA) or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of materials described in this text.

F. Quality Control

The performance of IBI FlexTube kits are regularly monitored. IBI FlexTube kits are tested by using them for extraction of Proteins, DNA and RNA fragments of various sizes from either agarose or Polyacrylamide gels. IBI FlexTube kits are also tested for simple dialysis of salts or buffer exchange. The quality of the isolated Protein, DNA and RNA fragments, or of the sample after dialysis is checked by several assays commonly used for proteins, nucleic acids, and dialysis. Determining the recovery from a specific amount of loaded samples will test the quality of the IBI FlexTube membranes.

G. IBI FlexTubes

These devices combine two modes of action, electro-elution of macromolecules from polyacrylamide or agarose gels and dialysis or buffer exchange of small volume samples (50-800µl). These devices allow rapid and high performance at either mode, and extract the macromolecules without any contamination. They are ideal for purification of very small quantities of proteins (up to 0.5µg), for automated protein sequencing, peptide mapping, and amino acid analysis.

Yield of Molecule Recovery

DNA or RNA from agarose gel	90%
DNA or RNA from polyacrylamide gel	90%
Protein from SDS-PAGE	70%

Specifications

Membrane cut-off	1K, 3.5K, or 6-8K MWCO
Tube volume capacity	800µl
Minimum amount of protein at the start of extraction	0.5µg
Maximum size of the gel slice that can be inserted into the tube	1cm x 0.5cm
Volume of sample for dialysis	50-800µl
Membrane	Ultra-clean, sulfur and heavy metal free. EDTA treated

H. Protein Extraction from Polyacrylamide Gels

IMPORTANT: 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, SeeBand Extraction Grade Protein Stain (IB01035), is good staining reagent as it will permanently stain the gel without undue fixing of the protein. Stain gel with SeeBand protein stain (IB01035) until band of interest is visible. Be careful not to stain gel for too long, most proteins require 1 to 5 minutes.

PROCEDURE

1. Fill the IBI FlexTube with 0.8ml of dH_2O, incubate for at least 5 minutes, and empty the tube.

   IMPORTANT: Check carefully that there is no dH_2O leaking from the tube. Absorption of water by the dry membrane may cause a decrease in water level.

2. After staining the gel (with SeeBand Protein Staining Solution), remove the gel slice containing the protein fragment with a clean, sharp scalpel.

   Minimize the size of the gel slice by removing extra gel.

3. Transfer the gel slice into an IBI FlexTube. Fill the tube with protein-running buffer (0.7-0.8µl). Close the tube gently.

   Avoid introducing air bubbles into the tube. The maximum size of the gel slice per IBI FlexTube is 1 x 0.5cm; do not fill the tube with more than 1 gel slice, for larger gel slices use more than one IBI FlexTube.

4. Place the IBI FlexTube in the provided supporting tray (see Figure 1).

   The supporting tray can hold 1 - 4 IBI FlexTube(s).

   IMPORTANT: The arrow on the cap should be pointing face up. The two membranes of the IBI FlexTube must be in perpendicular to the electric field to permit the electric current to pass through the tube.

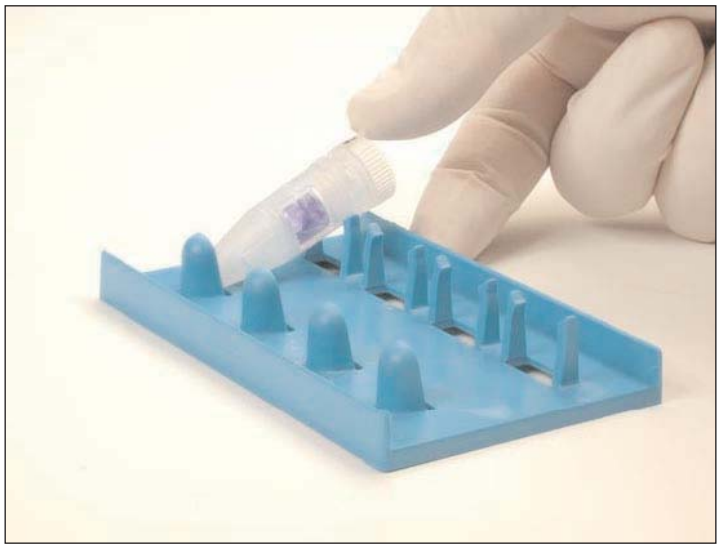

Figure 1: Insertion of the IBI FlexTube in the provided supporting tray. The arrow on the cap should be pointing upwards.

5. Place the supporting tray containing the IBI FlexTube(s) in an IBI horizontal electrophoresis tank containing a protein running buffer (see Figure 2).

   IMPORTANT: Fully immerse the IBI FlexTube(s) in buffer prior to running.

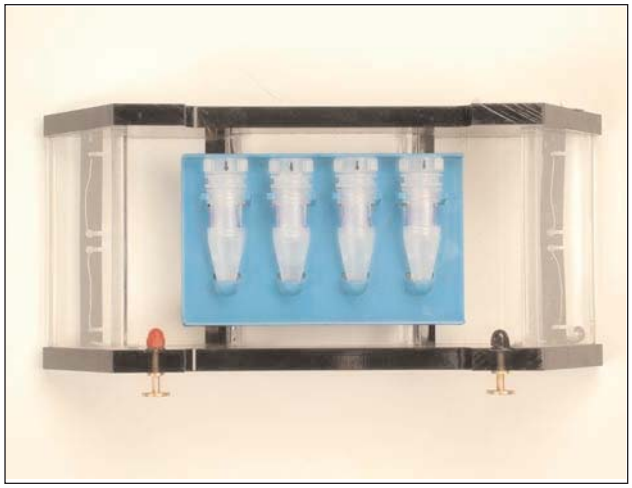

Figure 2: Shows the supporting tray containing four the IBI FlexTubes in an IBI QS710 horizontal electrophoresis tank. The arrow on the cap should be pointing upward and the two membranes of the IBI FlexTubes are in perpendicular to the electric field.

6. Run the gel (usually at 100 volts) until the protein exits from the gel slice.

   Electro-elution time is to be adjusted for each individual sample. It takes at least 85 minutes for BSA protein to be electro-eluted from a 10% SDS-PAGE slice (see Table 1).

7. Reverse the polarity of the electric current for 120 seconds.

   This step will release the protein from the membrane.

8. Open the IBI FlexTube gently, pipetting the protein-containing solution up and down carefully (at least 5 times) and transfer the solution to a clean 1.5ml microcentrifuge tube.

   Do the pipetting on the inner side of the membrane.

9. Centrifuge the microcentrifuge tube for 1 minutes at maximum speed.

   This step will remove gel residues.

10. Transfer the protein-containing solution to a clean 1.5ml microcentrifuge tube.

    Notes:

    i. Use the extracted protein directly.
    ii. Concentrate the extracted protein by standard concentration methods.
    iii. Concentrate the extracted protein.
    iv. Precipitate the extracted protein by standard precipitation protocols.
    v. Dialyze directly the extracted protein by using an IBI FlexTube

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. Electro-elution time at the elution step was to be adjusted for each individual sample.

Table 1: Minimum time needed to extract different-sized proteins from 10% SDS-polyacrylamide gel (29:1 polyacrylamide:bisacrylamide) at 100V.

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

I. Protein Precipitation Protocols

Trichloroacetic acid (TCA) precipitation procedure

1. Add equal volume of 20% TCA to the microcentrifuge tube containing the extracted protein solution and mix properly.

   For example, add 700µl of 20% TCA to a 700µl sample.

2. Incubate for 1 hour at 4°C.
3. Spin in a microcentrifuge at 4°C for 30 minutes at 14,000RPM.
4. Discard supernatant carefully.
5. Add 500µl of cold acetone.
6. Incubate at -20°C for 30 minutes and centrifuge the sample at 4°C for 30 minutes at 14,000RPM.

   To increase protein precipitation yield incubate the samples over night at -20°C.

7. Discard supernatant and air-dry the pellet.
8. Resuspend the pellet using 0.1M NaOH or dH_2O (use at least 20µl to perform resuspension). If dH_2O is used for resuspension, incubate the sample for 5 minutes at 60°C, resuspend the sample and incubate 5 minutes more at 60°C.

MS precipitation procedure

1. Add 1:10 by volume of MS buffer to the protein containing solution and mix properly.

   For example, add 70µl of MS buffer to a 700µl sample.

2. Incubate for 15 minutes at room temperature.
3. Add 1:2 by volume of 20% TCA and mix properly.

   For example, add 385µl of 20% TCA to a 770µl sample.

4. Incubate for 1 hour at 4°C.
5. Centrifuge the sample at 4°C for 30 minutes at 14,000RPM.
6. Carefully descent the supernatant without disturbing the pellet.
7. Add 500µl of cold acetone.

8. Incubate at -20°C for 30 minutes and centrifuge the sample at 4°C for 30 minutes at 14,000RPM.

   To increase protein precipitation yield incubate the samples over night at -20°C.

9. Carefully descent the supernatant without disturbing the pellet. Air-dry the pellet.

10. Resuspend the pellet in a suitable buffer solution or 0.1M NaOH (use at least 20µl to perform resuspension).

J. Protein Extraction from Polyacrylamide Gel compatible with Matrix-assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS) using IBI FlexTubes

Introduction

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is a widely used technique for the separation and molecular weight estimation of individual proteins. However, the accuracy of this molecular weight determination is often inadequate for protein characterization. More recently Matrix-assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-TOFMS) has found widespread use for the determination of molecular mass of intact proteins isolated from gels. The isolation of proteins from gels with the newly developed IBI FlexTube electro-elution system has over 80% recovery yields. This combination of SDS-PAGE, IBI FlexTube electro-elution system and MALDI-TOFMS is attractive. It provides a much more accurate determination of protein molecular weight. Moreover, even difficult proteins to analyze such as integral membrane proteins (hydrophobic) or high molecular mass proteins can be analyzed. This unique method provides a powerful means for characterizing endogenous proteins of wide molecular weight range separated by SDS-PAGE.

The combination of the three methods provides significantly improved protein yield and SDS free samples. The end result is a MALDI-MS analysis with greater sensitivity. The IBI FlexTube tool provides high protein yield recovery, and the MS buffer contained in the IBI FlexTube kit thoroughly removes the SDS.

Procedure

1. Fill the IBI FlexTube with 0.8 ml of dH_2O; incubate for at least 5 minutes. Empty the tube.

   IMPORTANT: Check carefully that no dH_2O is leaking from the tube. Absorbent of water, by the dry membrane, cause to the decrease in water level.

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

   Minimize the size of the gel slice by removing extra gel. Using the SeeBand staining solution will result in the best recovery yield of proteins from the gel.

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

Avoid air bubbles in the tube. The maximum size of gel slice per IBI FlexTube is 1cm x 0.5cm; don't fill the tube with several gel slices; for large gel slices use more than one tube.

4. Place the IBI FlexTube in the provided support tray (see Figure 1).

   The support tray can hold 1-4 IBI FlexTube(s).

5. Place the support tray containing the IBI FlexTube(s) in a horizontal electrophoresis tank filled with protein-running buffer (250mM Tricine pH 8.5, 0.025% SDS and 25mM Tris-Base) (see Figure 2).

   IMPORTANT: Immerse fully the IBI FlexTube(s) with the tray in the buffer.

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

   The electro-elution time is to be adjusted for each individual sample. It takes at least 2 hours for BSA protein to be electro-eluted from a 10% SDS-PAGE gel slice.

   Increase electro elution time in Table 1 page 9 by 30% .

7. Reverse the polarity of the electric current for 120 seconds.

   This step will remove the protein from the membrane.

8. Open the IBI FlexTube gently, pipetting the protein-containing solution up and down carefully (at least 5 times) and transfer the solution to a clean 1.5ml microcentrifuge tube.

   Do the pipetting on the inner side of the membrane.

9. Centrifuge the microcentrifuge tube for 1 minute at maximum speed.

   This step will remove gel residues.

10. Transfer the protein-containing solution to a clean 1.5ml microcentrifuge tube.

PRECIPITATION PROTOCOL OF PROTEIN FOR ANALYSIS BY MALDI-MS

1. Add 1:10 by volume of MS buffer to the protein containing solution and mix properly.

For example, add 70µl of MS buffer to a 700µl sample.

2. Incubate for 15 minutes at room temperature.
3. Add 1:5 by volume of 50% TCA (not provided in the kit) and mix properly.

   For example, add 154µl of 50% TCA to a 770µl sample.

4. Incubate for 1 hour at 4°C.
5. Centrifuge the sample at 4°C for 30 minutes at 14,000RPM.
6. Carefully descent the supernatant without disturbing the pellet.
7. Add 500µl of ice-cold acetone.
8. Incubate at -20°C for 30 minutes and centrifuge the sample at 4°C for 30 minutes at 14,000RPM.

   To increase protein precipitation yield incubate the samples over night at -20°C.

9. Carefully descent the supernatant without disturbing the pellet. Air-dry the pellet.
10. 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 20µl to perform resuspension.

K. Dialysis with the IBI FlexTube

Procedure

1. Fill the IBI FlexTube with 0.8ml of dH_2O; incubate for at least 5 minutes. Empty the tube.

   IMPORTANT: Check carefully that there is no dH_2O leaking from the tube. Absorbent of water, by the dry membrane, cause to the decrease in water level.

2. Load sample into the IBI FlexTube. Close the tube.

   Sample volume should be in the range of 50-800µl. If small volume is used, load the sample close to the inner membrane.

3. Place the loaded IBI FlexTube in the supplied floating rack in a stirred beaker containing large volume (usually at least 100X that of the sample) of the desired buffer.

   The floating rack can hold 1-4 IBI FlexTube(s).

   Adjust the stir bar speed. Allow at least 30 minutes for each 0.1ml of sample. Low-molecular weight salts and buffers (e.g., Tris·Cl and KPO_4) equilibrate within 3 hours. Equilibration times for viscous samples will be longer.

   IMPORTANT: The user must determine exact equilibration times for the dialysis.

4. Change the dialysis buffer as necessary.

5. Pipette out the sample carefully from the IBI FlexTube to a clean microcentrifuge tube.

   If sample volume increased during dialysis, let your sample evaporate on the bench top (using a fan to increase airflow across the membrane will speed up the process), making sure to check every 10 minutes or less to prevent evaporation to dryness.

L. Sample Concentration by Evaporation with IBI FlexTube

IBI FlexTubes are ideally suited for sample concentration via evaporation because of their dual membranes and large surface area. Dialysis and concentration in the same device reduce protein loss. Unlike closed-system centrifuge-type devices, sample concentration can be easily monitored in the IBI FlexTubes.

1. Place a sample in the IBI FlexTube or use already dialyzed sample and place it on microtube rack stand.

2. Let your sample evaporate on the bench top (using a fan to increase airflow across the membrane will speed up the process), making sure to check every 10 minutes or less to prevent evaporation to dryness. When concentrating by evaporation the water from your sample, the small molecule (buffer salts, reducing agents, etc.) will also be concentrated because no diffusion occurs.

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

M. DNA & RNA Extraction from Gels w/IBI FlexTubes

This procedure is designed to extract DNA or RNA from polyacrylamide or agarose gels.

Procedure

1. Fill the IBI FlexTube with 0.8ml of dH_2O, incubate for at least 5 minutes. Empty the tube.

   IMPORTANT: Check carefully that no dH_2O 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.

3. Transfer the gel slice to a IBI FlexTube. Fill the tube with dH_2O (0.7-0.8ml). Close the tube gently.

   Avoid air bubbles in the tube. The maximum size of gel slice per IBI FlexTube is 1cm x 0.5cm; don't fill the tube with several gel slices, for large gel slices use more than one tube.

4. Place the IBI FlexTube in the provided tray (see Figure 1).

   The supporting tray can comprise 1-4 IBI FlexTube(s).

   IMPORTANT: The arrow on the cap is pointing face up. The two membranes of the IBI FlexTube must be in par perpendicular to the electric field to permit the electric current to pass through the tube.

5. Place the supporting tray containing the IBI FlexTube(s) in a horizontal electrophoresis tank containing running buffer (see Figure 2).

   IMPORTANT: Immerse fully the IBI FlexTube(s) with the tray in the buffer.

   The two membranes of the IBI FlexTube must be in perpendicular to the electric field to permit the electric current to pass through the tube.

6. Pass electric current (usually at 80-150 volts) until the nucleic acid exits from the gel slice (see Tables 2 and 3).

   Optional: Follow the DNA or RNA eluted out of the gel with a hand-held UV lamp or table.

   IMPORTANT: The electro-elution time needs to be adjusted for each individual sample.

7. Reverse the polarity of the current for 120 seconds.

   This step will release the nucleic acid from the membrane.

8. Open the IBI FlexTube gently, pipetting the solution up and down carefully (at least 5 times) and transfer the solution to a clean 1.5ml microcentrifuge tube.

   Do the pipetting on the inner side of the membrane.

9. Centrifuge the microcentrifuge tube for 1 minutes at maximum speed.

   This step will remove gel residues.

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

    Note: Concentrate the extracted nucleic acid by standard concentration methods; for nucleic acid precipitation.

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.

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

Table 2: 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 (bp)**	**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 3: Minimum time needed to extract DNA fragments from 1% agarose gel at 80-110 volts.

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

N. DNA or RNA Precipitation

Procedure

1. Add 0.1 by volume of 3M KAc and 0.7-1 by volume of isopropanol to the solution. Mix gently by inverting the tube several times.

   For example, add 70µl of 3M KAc and 500-700µl isopropanol to a 700µl sample.

   Note: Addition of carrier (e.g. 20µg tRNA or 20µg glycogen) to the solution will increase the efficiency of precipitation.

2. Incubate at -20°C for 10 minutes.
3. Centrifuge the sample at 4°C for 30 minutes at 14,000RPM.
4. Carefully discard the supernatant without disturbing the pellet.
5. Wash the pellet with 0.5ml of cooled 70% ethanol.
6. Centrifuge at 4°C for 5 minutes at 14,000RPM.

Centrifuge the tube in the same orientation as previously to recover the DNA or RNA in a compact pellet.

7. Air-dry the pellet for 5-20 minutes

   Do not overdry 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.

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

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

O. Oligo PAGE Purification

This procedure is designed to extract oligo from polyacrylamide gels.

Before Starting

1. Separate the oligo on a sequencing PAGE in 1X TBE denatured gel.

Procedure

1. Fill the IBI FlexTube with dH_2O, incubate for at least 5 minutes. Empty the tube.

   IMPORTANT: Check carefully that no dH_2O is leaking from the tube. Absorption of water by the dry membrane may cause a decrease in water level.

2. Excise the slice of gel containing the desirable oligo fragment with a clean, sharp scalpel.

   Minimize the size of the gel slice by removing the extra gel.

3. Transfer the gel slice to an IBI Flextube. Fill the tube with dH_20.

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

4. Place the IBI Flextube(s) into the support tray, see figure 3.

   IMPORTANT: The two membranes of the IBI Flextube(s) must be in perpendicular to the electric field to permit the electric current to pass through the tube.

Figure 3: Insertion of the IBI FlexTube in the provided supporting tray. The arrow on the cap should be pointing upwards.

5. Place the support tray containing the IBI Flextubes in a horizontal electrophoresis tank, containing 1X TBE running buffer

   IMPORTANT: Immerse the IBI Flextube(s) completely in buffer.

6. Set the electric current to 500V for 15 minutes.

   Optional: Follow the DNA oligo eluted out of the gel with a hand-held UV lamp, by looking for the shadow created by the oligo on the florescent screen.

7. Reverse the polarity of the current for 3 minutes.

   This step will release the oligo from the membrane.

Desalting of the Sample

Open the IBI Flextube(s) gently.

Option 1. Desalting using desalting column: pipetting the solution inside the IBI Flextube up and down carefully (at least five times) and transfer the solution to a clean desalting column. Desalt the sample according to the desalting column protocol. This protocol will take approximately 15 minutes.

Option 2. Desalting by dialysis using IBI Flextube(s): remove the gel slice from the tube with pincettes (see important note below). Place the IBI Flextube(s) containing the sample to dialyze against a large volume of water. Dialyze at 60°C for 100 minutes.

IMPORTANT: To eliminate cross contamination while removing gel slices among different oligos, clean the pincette between on IBI Flextube and the next one.

P. Troubleshooting Guide

Low yield	Insufficient elution time	Increase elution time. Increase applied voltage.
	Current polarity was not reversed	Reverse the polarity of the current for 60 second.
	Incomplete emptying of the tube from the macromolecules-containing solution	Make sure to empty all the macromolecules containing solution at the end of elution.
	Ineffective precipitation	Use suitable precipitation procedures.
	Tube not fully immersed in the buffer of the electrophoresis tank	Fully immerse the tube in the buffer of the electrophoresis tank, using the supporting tray.
	Gel slice not fully immersed in the buffer inside the tube	Fully immerse the gel slice in buffer inside the tube
	More than one gel slice into the tube	Don't fill the tube with several gel slices, for large gel slices use more than one tube
	The electric current doesn't pass through the tube	The two membranes of the IBI FlexTube must be parallel to the electric field
Long elution time	Low applied voltage	Increase applied voltage.
	Gel slice is not fully immersed in the buffer inside the tube	Fully immerse the gel slice in buffer inside the tube
	Tube not fully immersed in the buffer of the electrophoresis tank	Fully immerse the tube in the buffer of the electrophoresis tank, using the supporting tray.
Macromolecules containing solution reduced after elution	Membrane not wetted before elution	Wet the membrane for 5 minutes with dH_2O before elution
	Pinhole in the membrane, due to careless handling of the tube	Change tube
Presence of air bubbles in the tube	Insufficient dH_2O or running buffer inside the tube	After inserting the gel slice in the tube, fill the tube to the top of the electro-elution windows.

IBI FlexTubes are manufactured by Gene-Bio-Application Ltd. - Israel

GeBaFlexTubes are covered by patent application WO0190731