FLEXTUBE HANDBOOK

MEGA

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

IBI/GEBA FLEXTUBES ARE COVERED BY PATENT APPLICATION WO0190731

IBI SCIENTIFIC

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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

- Dialysis or buffer exchange with volumes between 3 and 20ml.
- Preparation of protein samples for MALDI-MS,
- Sample concentration.
- Large-scale protein dialysis, such as antibodies and recombinant protein purification.
- Removal of contaminating micro-molecules.
- Tissue culture extraction purification.
- Removal of salts, surfactants, solvents, and detergents.
- Complex formation studies (protein-protein, protein-DNA, and protein-RNA).
- pH and buffer adjustment of sample solutions, protein extraction or cell extraction.
- High throughput dialysis.
- Peptide dialysis, as small as 10 amino acids.
- Virus-particles purification.

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 can be used for dialysis or buffer exchange at volume samples between 3 and 20ml. IBI FlexTubes allow rapid, secure, simple loading and recovery, with high performance as the most convenient, user friendly dialysis system on the market.

Specifications

Membrane cut-off	1K, 3.5K, 6-8K, 12-14K MWCO
Tube volume capacity	10, 15, or 20ml - depending on kit
Volume of sample for dialysis	3 - 20ml
Membrane	Ultra-clean, sulfur and heavy metal free. EDTA treated

H. Dialysis with MEGA FlexTube Kits

IMPORTANT: To perform dialysis with a range of 3 - 10ml use IBI MEGA 10ml FlexTubes. To perform dialysis with a rnage of 10 - 15ml us IBI MEGA 15ml FlexTubes. To perform dialysis with a range of 15 - 20ml use IBI MEGA 20ml FlexTubes.

Figure 1: Dialysis with MEGA FlexTube

Procedure

1. Fill the IBI MEGA FlexTube with 20ml 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. Avoid positioning the MEGA FlexTubes on their cap when containing samples.

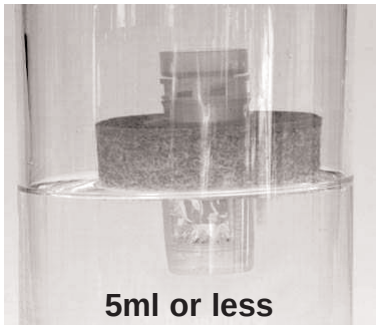
2. Load sample into IBI MEGA FlexTube. Close the tube with provided cap. For sample volume up to 10ml use MEGA 10ml FlexTubes. For sample volume between 10 and 15ml use MEGA 15ml FlexTubes. For sample volumes between 15 and 20ml use MEGA 20ml FlexTubes.

IMPORTANT: Sample volume should be in the range of 3 - 20ml. If smaller volumes (3-5ml) are used, load the samples close to the inner membrane.

Figure 2: 3 sizes of MEGA FlexTubes 10, 15, 20ml

3. Load IBI MEGA FlexTube into float. Position in beaker and stir. Volumes of the desired buffer are typically 100 to 1000 times that of the sample.

IMPORTANT: Adjust height of IBI MEGA FlexTube in the floating balance rack for upright floating during process. For less 5ml samples, place the floating rack up to the middle of the tube. For dialysis of more than 5ml, adjust the floating rack at the top of the MEGA FlexTube.



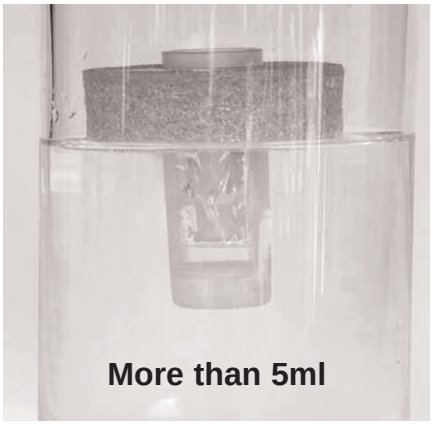


Figure 3: MEGA FlexTube floating in beaker

4. Adjust the stir bat speed. Low-molecular weight salts and buffers equilibrate within 3 hours. Equilibration time for viscous samples will be slightly longer.

IMPORTANT: User must determine exact equilibration times for the dialysis.

5. Change the dialysis buffer as necessary.

6. Pipette out the sample carefully from the IBI MEGA FlexTube into a clean tube.

   If sample volume has increased during dialysis, let your sample evaporate on the bench top (increasing airflow across the membrane will help speed up the process). Check the sample approximately every 10 minutes or less to prevent full evaporation and/or dryness.

Sample Concentration by Evaporation

IBI MEGA 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 reduces protein loss. Unlike closed-system centrifuge type devices, sample concentration can be easily monitored in the MEGA FlexTubes as well.

1. Place sample in the MEGA FlexTube or use an already dialyzed sample.
2. Let the sample evaporate on the bench top, using a fan to increase airflow across the membranes and speed up the process. Check the sample every 10 minutes of less to prevent full evaporation and dry sample condition. Concentrating by evaporation water with sample, small molecules (buffer salts, reducing agents, etc.) will also get concentrated because no diffusion occurs.

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

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 20ml of 20% TCA to a 20ml 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 14ml 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 0.7ml to perform resuspension).

MS precipitation procedure

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

   For example, add 0.3ml of MS buffer to a 3ml sample.
2. Incubate for 15 minutes at room temperature.
3. Add 1:2 by volume of 20% TCA and mix properly.

   For example, add 11ml of 20% TCA to a 22ml 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 14ml 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 0.7ml to perform resuspension).

J. DNA OR RNA PRECIPITATION

PROCEDURE

1. Add 0.1 by volume of 3M KAc pH-5.2 and 0.7-1 by volume of isopropanol to the solution. Mix gently by inverting the tube several times.

   For example, add 2ml of 3M KAc and 2.31 - 3.3ml isopropanol to a 14ml sample.

   Note: Addition of carrier (e.g. 80µg tRNA or 80µg 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 cooled 70% ethanol.
6. 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.
7. 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.

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