

DiaEasy™ Dialyzer (20 ml) MWCO 6-8 kDa

(Catalog # K1003-10, -25, -100; Store at Room Temperature (15-25°C); Shelf Life: 36 months)

Rev 09/13

I. Introduction:

Biovision's DiaEasy™ dialyzer (6-8 kDa MWCO) can be used primarily for dialysis or buffer exchanges of sample volumes up to 20 ml. They can also be used for several other purposes as shown below. The DiaEasy™ tube's membrane is ultra-clean, sulfur and heavy metal free and EDTA treated which makes it suitable for molecular biology experiments. These DiaEasy™ tubes allow rapid, secure and simple loading and recovery. They are very high performance dialysis tubes and are the most convenient, user friendly dialysis system on the market. The sample quality after dialysis can be checked by several assays commonly used for proteins and nucleic acids.

II. Applications:

- Dialysis or buffer exchange of sample volumes up to 20 ml.
- Preparation of protein samples for MALDI-MS.
- Samples concentration.
- Large-scale protein dialysis such as antibodies and recombinant protein purification.
- Peptide dialysis as small as 10 amino acids.
- Virus-particle purification.
- Removal of contaminating micro molecules.
- Tissue culture extracts purification.
- Removal of salts, surfactants, solvents and detergents.
- Complex formation studies (protein-protein, protein-DNA and protein-RNA).
- pH and buffer adjustment of sample solution, protein extract or cell extract.
- High through put dialysis (using a single beaker with several DiaEasy™ tubes on one floating pad).

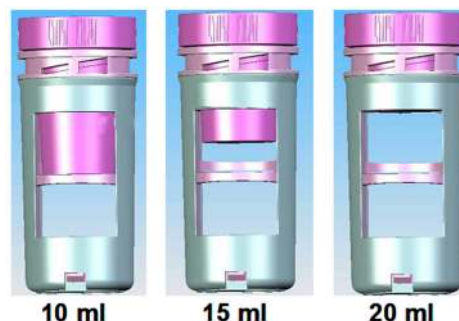


Figure 1: Dialysis with DiaEasy™ Dialyzer: Changing cap offers flexibility in the DiaEasy™ Dialysis tubes dialysis volumes that range from 3 to 20 ml.

III. Kit Contents:

Components	K1003-10	K1003-25	K1003-100	Part Number
DiaEasy™ Dialyzer Tubes	10	25	100	K1003-xx(x)-1
DiaEasy™ Dialyzer Floating Rack	2	5	10	K1003-xx(x)-2

IV. User Supplied Reagents and Equipment:

- Beakers and Buffers.

V. Storage and Handling:

Store all components of the kit at room temperature. Read the entire protocol before performing the experiment.

VI. Dialysis by DiaEasy™ Dialyzer Tubes:

To perform dialysis in the range of 15-20 ml use DiaEasy™ Dialysis tubes with the 20 ml cap.

Procedure:

- Fill the 20 ml DiaEasy™ Dialyzer tube with 20 ml of dH₂O. Incubate for at least 5 min. Then empty the tube.
 - IMPORTANT: Carefully check that no dH₂O leaking from the tube. Water absorption, by the dry membrane, causes decrease of water level.
 - Avoid positioning the 20 ml DiaEasy™ Dialysis tube on its cap when containing sample.
- Load sample into the 20 ml DiaEasy™ Dialyzer tube. Close the tube with the provided cap.
 - IMPORTANT: Sample volume should be in the range of 3-20 ml. If small volume (3-5 ml) is used (e.g., 3 ml), load the sample close to the inner membrane.
- Load 20 ml DiaEasy™ Dialyzer tube on floating rack. Position and stir in beaker containing larger volume (typically 100 to 1000-fold that of the sample volume) of desired buffer.
 - IMPORTANT: Adjust floating rack height on 20 ml DiaEasy™ Dialyzer tube for upright floating balance during dialysis process. Example: For dialysis of less than 5 ml place the floating rack upto middle the height of the 20 ml DiaEasy™ Dialyzer tube. For dialyses of more than 5 ml adjust the floating rack at the top of 20 ml DiaEasy™ Dialyzer tube (see Figure 2)
- 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.
 - IMPORTANT: User must determine exact equilibration times for the dialysis.
- Change the dialysis buffer as necessary.
- Pipette out the sample carefully from the 20 ml DiaEasy™ Dialyzer tube to a clean tube.
 - 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.

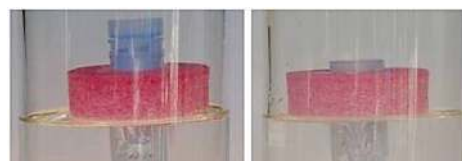


Figure 2: DiaEasy™ Dialysis floating rack in two positions to change the height at which the DiaEasy™ Dialysis tubes are held.

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- VII. Sample concentration by DiaEasy™ Dialyzer tubes:** DiaEasy™ Dialyzer tubes 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 DiaEasy™ Dialysis tubes.

Procedure:

- Place sample in the DiaEasy™ Dialyzer tubes or use already dialyzed sample.
- Let sample 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.
 - **IMPORTANT:** When evaporating water from your sample, small molecules (buffer salts, reducing agents, etc.) will also be concentrated.

VIII. Protein and Nucleic acid precipitation protocols used after isolation from the DiaEasy™ Dialyzer Tubes:

Trichloroacetic acid (TCA) precipitation procedure (for protein)

- Add equal volume of 20% TCA to the tube containing the extracted protein solution and mix properly. For example, add 20 ml of 20% TCA to a 20 ml sample.
- Incubate 60 min in 4°C.
- Centrifuge at 4°C for 30 min at 20,000 g and carefully discard the supernatant.
- Add 14 ml cold acetone and incubate at -20°C for 60 min.
- To increase protein precipitation yield incubate the samples overnight at -20°C.
- Centrifuge the sample at 4°C for 30 min at 20,000 g.
- Discard supernatant and air-dry the pellet.
- Resuspend the pellet in 0.1 M NaOH (use at least 0.7 ml to perform resuspension).

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

- Add 1:10 by volume of your choice of MS buffer to the protein containing solution and mix properly. For example, add 2 ml of MS buffer to a 20 ml sample.
- Incubate for 15 min at room temperature.
- Add 1: 2 by volume of 20% TCA and mix properly. For example, add 11 ml of 20% TCA to a 22 ml sample.
- Incubate for 1 hour at 4°C.
- Centrifuge the sample at 4°C for 30 min at 20,000 g and carefully decant the supernatant without disturbing the pellet.
- Add 14 ml of ice-cold acetone.
- Incubate at -20°C for 30 min and centrifuge the sample at 4°C for 30 min at 20,000 g.
- To increase protein precipitation yield incubate the samples over night at -20°C.
- Carefully decant the supernatant without disturbing the pellet. Air-dry the pellet.
- Resuspend the pellet in a suitable buffer solution or 0.1 M NaOH (use at least 0.7 ml to perform resuspension).

DNA or RNA precipitation procedure

- 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 2 ml of 3 M Potassium acetate pH-5.2 and 14 ml isopropanol to a 20 ml sample.
- Mix gently by inverting the tube several times.
 - Note: addition of carrier (e.g. 80 µg tRNA or 80 µg glycogen) to the solution will increase the efficiency of precipitation.
- Incubate at -20°C for 10 min.
- To increase DNA or RNA precipitation yield incubate the samples overnight at -20°C.
- Centrifuge the sample at 4°C for 30 min at 20,000 g.
- Carefully discard the supernatant without disturbing the pellet.
- Wash the pellet with cold 70% ethanol.
- Air-dry the pellet for 5-20 min.
 - 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.
- 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.

IX. Related Products

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| DiaEasy™ Dialyzer (20 ml) MWCO 3.5 kDa (K1000-10, -25, -100) | DiaEasy™ Dialyzer (15 ml) MWCO 3.5 kDa (K1001-10, -25, -100) |
| DiaEasy™ Dialyzer (10 ml) MWCO 3.5 kDa (K1002-10, -25, -100) | DiaEasy™ Dialyzer (15 ml) MWCO 6-8 kDa (K1004-10, -25, -100) |
| DiaEasy™ Dialyzer (10 ml) MWCO 6-8 kDa (K1005-10, -25, -100) | DiaEasy™ Dialyzer (20 ml) MWCO 12-14 kDa (K1006-10, -25, -100) |
| DiaEasy™ Dialyzer (15 ml) MWCO 12-14 kDa (K1007-10, -25, -100) | DiaEasy™ Dialyzer (10 ml) MWCO 12-14 kDa (K1008-10, -25, -100) |
| DiaEasy™ Dialyzer (20 ml) MWCO 1 kDa (K1009-10, -25, -100) | DiaEasy™ Dialyzer (15 ml) MWCO 1 kDa (K1010-10, -25, -100) |
| DiaEasy™ Dialyzer (10 ml) MWCO 1 kDa (K1011-10, -25, -100) | |

FOR RESEARCH USE ONLY! Not to be used on humans.