# Pur–A–Lyzer™ Midi Dialysis Kit Manual

SIGMA-ALDRICH
Labware

SIGMA Life Science · ALDRICH Chemistry · Fluka Analytical · SUPELCO Analytical

SIGMA-ALDRICH®

## Pur–A–Lyzer™ Midi Dialysis Kit

| Catalog Numbers | | |
|---|---|---|
| PURD10005 | PURD35010 | PURD60010 |
| PURD35030 | PURD60030 | PURD35050 |
| PURD60050 | PURD35100 | PURD60100 |

## Product Description

Pur–A–Lyzer kits may be used for:

- Dialysis or buffer exchange
- Sample concentration by evaporation
- Protein/precipitation
- Nucleic acid concentration

Midi tube capacity: 50–800 µL

## Midi Pur–A–Lyzer Kit Contents

| Product Number | Molecular Wt. Cut–off | Tubes |
|---|---|---|
| PURD10005 | 1 kDa | 5 tubes |
| PURD35010 | 3.5 kDa | 10 tubes |
| PURD35030 | 3.5 kDa | 30 tubes |
| PURD35050 | 3.5 kDa | 50 tubes |
| PURD35100 | 3.5 kDa | 100 tubes |
| PURD60010 | 6-8 kDa | 10 tubes |
| PURD60030 | 6-8 kDa | 30 tubes |
| PURD60050 | 6-8 kDa | 50 tubes |
| PURD60100 | 6-8 kDa | 100 tubes |

Each kit includes a floating rack along with the Pur-A-Lyzer tubes (PURNR-1EA).

The regenerated cellulose membrane is ultraclean, sulfur and heavy metal free, and EDTA treated.

Pur-A-Lyzers are tested for simple dialysis of salts or buffer exchange. The quality of the sample after dialysis is checked by several assays. Determining the recovery from a specific amount of loaded sample also tests the quality and efficiency of the Pur-A-Lyzer membrane.

## Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Granted Patent related to Pur-A-Lyzer, (1). Australia 2001262612, (2). Canada 2410322, (3). Europe 1285257, (4) India 201731, (5) S Korea 823939, (6) USA 7074313.

## Storage/Stability

Store the Pur-A-Lyzer kit in a dry place at room temperature. Under these conditions, a kit can be stored for up to 12 months without any deterioration in performance and quality. For longer storage time, it is recommended the Pur-A-Lyzer kit be stored in a cool place (refrigerator), at a relative humidity of at least 35%.

## Procedures

Dialysis with Midi Pur-A-Lyzer
Sample concentration by evaporation
Protein precipitation
DNA/RNA concentration

## Dialysis with Midi Pur–A–Lyzer

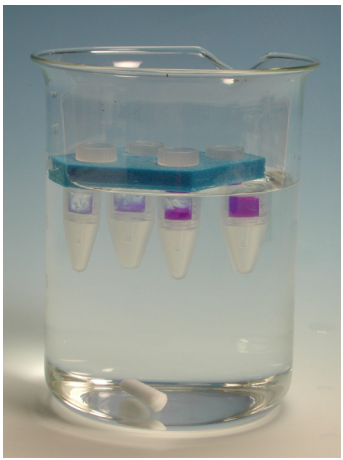

Figure 1: Dialysis with Midi Pur–A–Lyzer.

### Procedure

1. Fill the Pur–A–Lyzer with 0.8 mL of ultrapure water; incubate for at least 5 minutes. Empty the tube.

   **Note:** Check carefully that there is no water leaking from the tube. Absorption of water by the dry membrane will cause a decrease in water level.

2. Load sample into the Pur–A–Lyzer tube. Close the tube with the provided caps (do not apply force).

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

3. Place the loaded Pur–A–Lyzer tube in the supplied floating rack and then place the 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 Pur–A–Lyzer tubes.

   Adjust the stir bar speed. Allow at least 30 minutes for each 0.1 mL of sample. Low–molecular weight salts and buffers (e.g., tris and phosphate) equilibrate within 3 hours. Equilibration times for viscous samples will be longer.

   **Note:** The user must determine exact equilibration times for the dialysis.
4. Change the dialysis buffer as necessary.
5. Pipette the sample carefully from the Pur–A–Lyzer to a clean tube.

   If sample volume increased during dialysis, concentrate the sample by evaporation.

## Sample Concentration by Evaporation with Midi Pur–A–Lyzer

Pur–A–Lyzers kits are ideally suited for concentration of the sample by 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 Pur–A–Lyzers.

1. Place a sample in the Pur–A–Lyzer or use already dialyzed sample, and place it on micro tube rack stand.
2. Let the 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.

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

## Trichloroacetic acid (TCA) precipitation procedure

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

   For example, add 300 µL of 20% TCA to a 300 µL sample.
2. Incubate 60 minutes in 4 °C.
3. Spin the tube at 4 °C for 30 minutes at 14,000 rpm.
4. Discard supernatant carefully.
5. Add 500 µL of cold acetone.
6. Incubate at –20 °C for 60 minutes and centrifuge the sample at 4 °C for 30 minute sat 14,000 rpm.

   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 in appropriate buffer (use at least 20 µL to perform resuspension).

## DNA or RNA precipitation

1. Add 0.1 volume of 3 M potassium acetate, pH 5.2, and 0.7–1 volume of isopropanol to the solution. Mix gently by inverting the tube several times.

   For example, add 30 µL of 3 M potassium acetate pH 5.2, and 210–300 µL isopropanol to a 300 µL sample.

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

2. Incubate at –20 °C for 10 minutes.

   To increase DNA or RNA precipitation yield incubate the samples over night at –20 °C .

3. Centrifuge the sample at 4 °C for 30 minutes at 14,000 rpm.
4. Carefully discard the supernatant without disturbing the pellet.
5. Wash the pellet with 0.5 mL of a cooled 70% ethanol solution.
6. Air–dry the pellet for 5–20 minutes.

   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.

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.

*Enabling Science to*
*Improve the Quality of Life*

**Order/Customer Service (800) 325-3010 • Fax (800) 325-5052**
**Technical Service (800) 325-5832 • sigma-aldrich.com/techservice**
**Development/Custom Manufacturing Inquiries SAFC® (800) 244-1173**
**Safety-related Information sigma-aldrich.com/safetycenter**

**World Headquarters**
3050 Spruce St.
St. Louis, MO 63103
(314) 771-5765
**sigma-aldrich.com**



78103
1032

SIGMA-ALDRICH®