

Screening plate (Cat. #: XZ-S-96)

Optimization plate (Cat. #: XZ-O-96)

Growth and soaking plate (Cat. #: XZ-G-96)

Growth plate (Cat. #: XZ-G-24)

Introduction to the XZ™ plates.

Dialysis and the XZ™ plates.

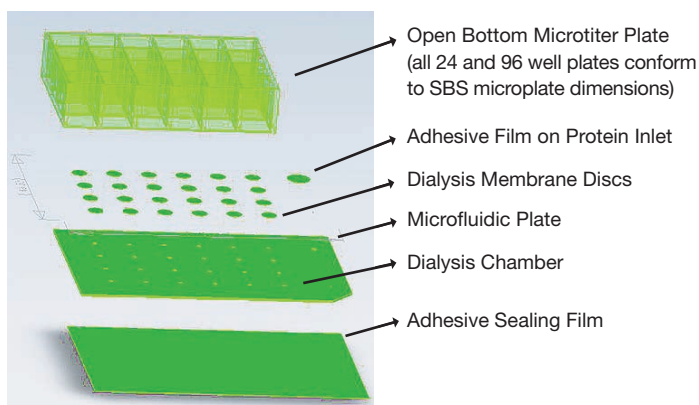
Dialysis protein crystallization is a well-established method for protein crystallization developed during the early days of structural biology. Dialysis is the only technique that allows crystallization of a biological macromolecule by either salting in or salting out. Although it is a proven crystallization method, that has been used to crystallize and solve the structure of hundreds of proteins (examples listed below), it has been labor intensive and required significant amounts of protein.

- **Aspartate carbamoyl transferase (Steitz et.al. PNAS)**
- **Poliovirus (Hogle et.al. Science)**
- **Theiler's murine encephalomyelitis virus (Grant et.al. PNAS)**
- **Liver alcohol dehydrogenase (Cho et.al. Biochemistry)**
- **Neuroaminidase (Vargese et.al. J.Mol.Bio.)**
- **L-arabinose binding protein (Quiocho et.al. Nature)**
- **Photosystem I reaction centre (Krauss et.al. Nature)**

The disposable XZ™ microfluidic dialysis technology has been developed to specifically address these issues. With the XZ™ microfluidic dialysis technology, dialysis protein crystallization trials as low as 15nL/reaction can be easily and quickly setup. This reaction volume represents a reduction in protein consumption per experiment that is > 3,000 times lower than the currently available dialysis products. Another major advantage of the XZ™ plates is its high-throughput compatibility with common liquid dispensing and imaging systems. In addition, the XZ™ microfluidic dialysis technology offers a clear path to structure due to straight forward scale-up designs, easy loop access for crystal harvesting, and in-situ x-ray diffraction capability.

XZ™ design

The XZ™ dialysis protein crystallography plate is composed of a microfluidic plate bonded to an open bottom microtiter plate. The microfluidic plate contains a microfluidic network on the bottom which delivers a protein sample solution to the dialysis chambers. The microfluidic plate is sealed on the bottom (microfluidic channel side) with a clear film and on the top with dialysis membrane discs over the dialysis chambers. The dialysis disc side of the microfluidic plate is then bonded to an open-bottom microtiter plate.



Dialysis Crystallization Screen – Salt-in, Salt-out™

Dialysis is the only crystallization technique that allows crystallization in both the salting in (low ionic strength) and salting out region (high ionic strength). Consequently, it is advantageous to use a crystallization screen that explores both traditional high ionic strength precipitants as well as dialysis specific low ionic strength precipitants. Microlytic has designed a unique patent pending dialysis crystallization sparse matrix screen Salt-in, Salt-out™ that thoroughly explores the protein phase diagram in both the salting in region and the salting out region. The screen is designed based on an analysis of crystallization conditions that have been successful in crystallizing by dialysis over 100 different targets ranging from integral membrane proteins, protein complexes, viruses, and soluble proteins.

If using other crystallization screens for the XZ™ plates, note that PEGs with MW above 8000 and PEG concentrations above 10 % w/v may result in sub-optimal equilibration across the dialysis membrane and should be used with caution.

Equipment and accessories needed for XZ™ plate set up:

- (1) Vacuum pump (Cat. #: XZ-VP) or in house vacuum line with an ultimate vacuum less than 2mmHg (equal to 2 torr = 2.7 mbar equal to 29.8 inHg)
- (2) Vacuum pump plate connector (Cat. #: XZ01-01). The connectors on the vacuum pump connector are of different size and the smaller connector fits the 5 mm diameter vacuum port on the XZ™ plate, whereas the larger connector fits into a 6.5 mm (1/4") I.D. tubing from the vacuum source.
- (3) Disposable needle (Cat. #: XZ01-02)
- (4) Purge liquid (Cat.#: XZ01-05)
- (5) Dialysis crystallization screen (Cat#: MLY-5D) or equivalent

Experienced user protocol.

1. Load **75 uL (XZ-S-96) / 75 uL (XZ-O-96) / 200 uL (XZ-G-96) / 500 uL (XZ-G-24)** precipitant solution to each of the XZ™ plate wells
2. Apply vacuum to XZ™ device
3. Load **6 uL (XZ-S-96) / 15 uL (XZ-O-96) / 36 uL (XZ-G-96) / 25 uL (XZ-G-24)** protein sample onto sample inlet film
4. Introduce protein sample into XZ™ plate
5. Purge XZ™ plate with **15 uL (XZ-S-96) / 30 uL (XZ-O-96) / 72 uL (XZ-G-96) / 50 uL (XZ-G-24)** purge solution
6. Inspection of dialysis experiments
7. Harvesting crystals from growth plates
8. In situ diffraction crystal quality determination

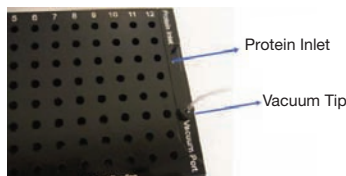
Detailed protocol for setting up the XZ™ plates.

1. Load 75 μ L (XZ-S-96) / 75 μ L (XZ-O-96) / 200 μ L (XZ-G-96) / 500 μ L (XZ-G-24) precipitant solution to each of the XZ™ plate wells



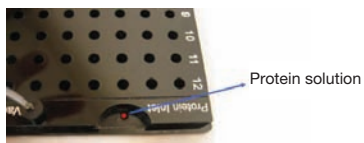
- Using a single or multichannel pipette load the appropriate precipitant solution volume to each of the wells.
- Seal off precipitant inlets without covering the vacuum port and the protein inlet or vacuum port. Alternatively, the XZ™ plate can be sealed after step 6 in protocol.

2. Apply vacuum to XZ™ device



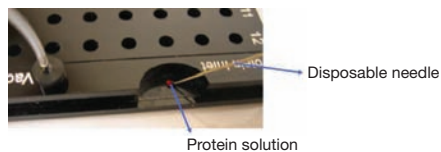
- Turn on vacuum pump (Cat. #: XZ-VP) or in house vacuum line with an ultimate vacuum less than 2mmHg (equal to 2 torr = 2.7 mbar equal to 29.8 inHg).
- Connect vacuum pump connector (Cat. #: XZ01-01) to vacuum source. Note that connectors on vacuum pump connector are of different size. The larger connector fits into a 6.5 mm (1/4") I.D. tubing from the vacuum source and the smaller connector fits the 5 mm diameter vacuum port on the XZ™ plate.
- Connect vacuum pump plate connector (Cat. #: XZ01-01) to XZ™ plate. **Make sure all connections are tight, since leaky connections may result in air bubbles forming in all dialysis chambers.**
- Start a timer to allow for 1 minute evacuation of air from the XZ™ dialysis plate and proceed immediately to step 3.

3. Load 6 μ L (XZ-S-96) / 15 μ L (XZ-O-96) / 36 μ L (XZ-G-96) / 25 μ L (XZ-G-24) protein sample onto sample inlet film



- During the 1 minute vacuum evacuation, pipette the appropriate volume of protein sample onto the protein inlet film. Make sure that the protein solution is bubble free and centered on the protein inlet. Proceed immediately to step 4.

4. Introduce protein sample into XZ™ plate

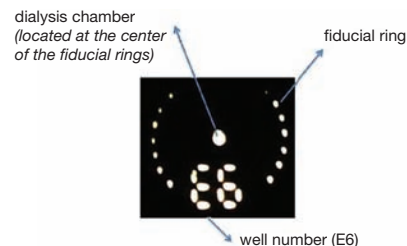


- At the 1 minute mark of vacuum evacuation, puncture the protein inlet film below the protein drop using a disposable needle (Cat. #: XZ01-02). The dialysis chambers will be filled with the protein sample due to the vacuum force.
- Maintain vacuum on the XZ™ plate for 2 minutes from the time the protein inlet film was punctuated.

5. Purge XZ™ plate with 15 μ L (XZ-S-96) / 30 μ L (XZ-O-96) / 72 μ L (XZ-G-96) / 50 μ L (XZ-G-24) purge solution

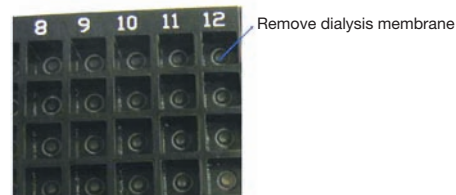
- Pipette the appropriate amount of purge liquid (Cat.#: XZ01-05) to the pierced part of the protein inlet film. The purge solution will flush out excessive protein solution in the microfluidic channel network and ensure complete isolation of the dialysis chambers.
- Maintain vacuum for 2 min after the addition of the purge liquid.
- Disconnect vacuum pump connector.
- Add 15 μ L of purge liquid to both protein inlet and vacuum port.

6. Inspection of dialysis experiments



- Dialysis experiments can be inspected using a stereomicroscope using base/transmitted illumination, using an inverted microscope in transmitted light mode or using an automated imaging system.
- For the screening plate (Cat. #: XZ-S-96), optimization plate (Cat. #: XZ-O-96), and growth and soaking plate (Cat. #: XZ-G-96) fiducial rings and well numbers are positioned on the bottom of the wells to help locate and identify the dialysis chambers.

7. Harvesting crystals from growth plates. Applies to growth and soaking plate (Cat. #: XZ-G-96) and growth plate (Cat. #: XZ-G-24).



- Using a pair of tweezers, the dialysis membrane can be removed from the bottom of the precipitant well while precipitant solution is still present.
- Cryo-solution may be added prior to removal of the dialysis membrane; however, note that PEGs should be avoided as cryo solvents, if added while dialysis membrane is still present, as they may dehydrate the dialysis chamber.
- Crystals can now be harvested from the dialysis chamber using standard loops.

8. In situ diffraction crystal quality determination

- XZ™ plates can be directly mounted in a plate holder at a beamline and crystal quality can be evaluated in situ. Approximate degree of rotation is listed in table below.

XZ™ Plate	Degree rotation
Screening plate (Cat. #: XZ-S-96)	+/- 20 degrees
Optimization plate (Cat. #: XZ-O-96)	+/- 20 degrees
Growth and soaking plate (Cat. #: XZ-G-96)	+/- 35 degrees
Growth plate (Cat. #: XZ-G-24)	+/- 50 degrees