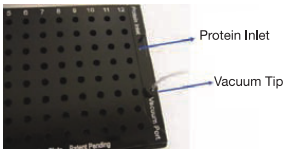
Detailed protocol for setting up the XZ™ plates.

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

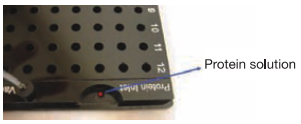
- Using a single or multichannel pipette load the appropriate precipitant solution volume to each of the wells.
- Seal off precipitant inlets without covering the vacuum port and the protein inlet or vacuum port. Alternatively, the XZ™ plate can be sealed after step 6 in protocol.

2. Apply vacuum to XZ™ device



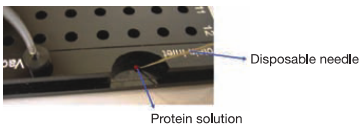
- Turn on vacuum pump (Cat. #: XZ-VP) or in house vacuum line with an ultimate vacuum less than 2mmHg (equal to 2 torr = 2.7 mbar equal to 29.8 inHg).
- Connect vacuum pump connector (Cat. #: XZ01-01) to vacuum source. Note that connectors on vacuum pump connecter are of different size. The larger connector 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 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



- 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

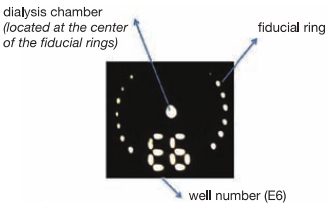


- At the 1 minute mark of vacuum evacuation, puncture the protein inlet film below the protein drop using a disposable needle (Cat. #: XZ01-02). The dialysis chambers will be filled with the protein sample due to the vacuum force.
- Maintain vacuum on the XZ™ plate for 2 minutes from the time the protein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

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

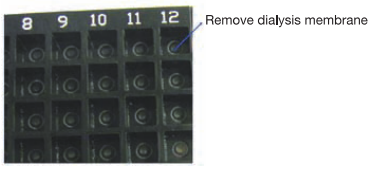
- Pipette the appropriate amount of purge liquid (Cat.#: XZ01-05) to the pierced part of the protein inlet film. The purge solution will flush out excessive protein solution in the microfluidic channel network and ensure complete isolation of the dialysis chambers.
- Maintain vacuum for 2 min after the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

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