

A Step-by-Step Procedure for Using XZ™ 1 × 1 and 1 × 4 Growth Plates

GN Biosystems, Inc.

Key Parameters

Protein sample volume to load the dialysis chamber(s): 1.5µl for 1x1 plates, and 4.5µl for 1x4 plates.

Reagent volume: 500µl/well

Equipment and Accessories Needed

- Vacuum pump or house vacuum with an ultimate vacuum $\leq 2\text{mmHg}$ (= 2 torr = 2.7mbar = 29.8inHg)**

**Air bubbles in every dialysis chamber will result due to insufficient vacuum*



Weight: 4lbs (1.8kg)

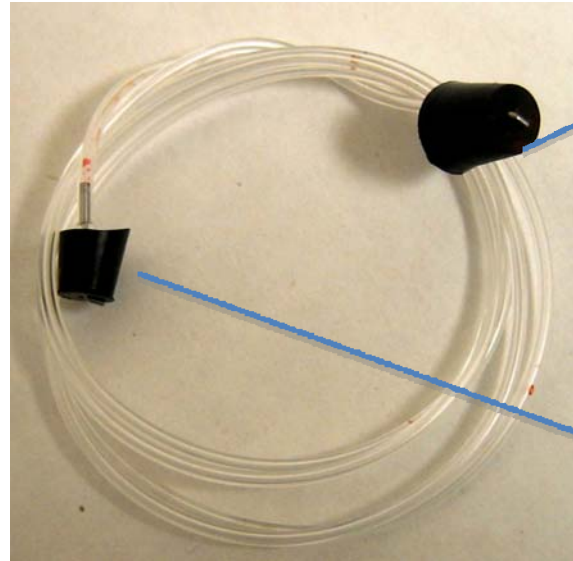
A List of Qualified Vacuum Pumps at under \$2,000

Manufacture	Model#	Ultimate Vacuum
KnF	N84.4	1.5mmHg
BOC/Edwards	RV3	$1 \times 10^{-6}\text{mmHg}$
BOC/Edwards	EVA480-16	0.008mmHg
Welch	1400B-01	$1 \times 10^{-4}\text{mmHg}$
Welch	1399B-01	0.02mmHg
Brinkmann	V-500	0.01mmHg
Thermo-Electron	3178712	$3.8 \times 10^{-3}\text{mmHg}$
Thermo-Electron	3178707	$1 \times 10^{-4}\text{mmHg}$



Equipment and Accessories Needed

2. Vacuum tip with vacuum tube adaptor (part# XZ01-01)



Vacuum tube adaptor: fit 1/4" (6.5mm) I.D. tubing

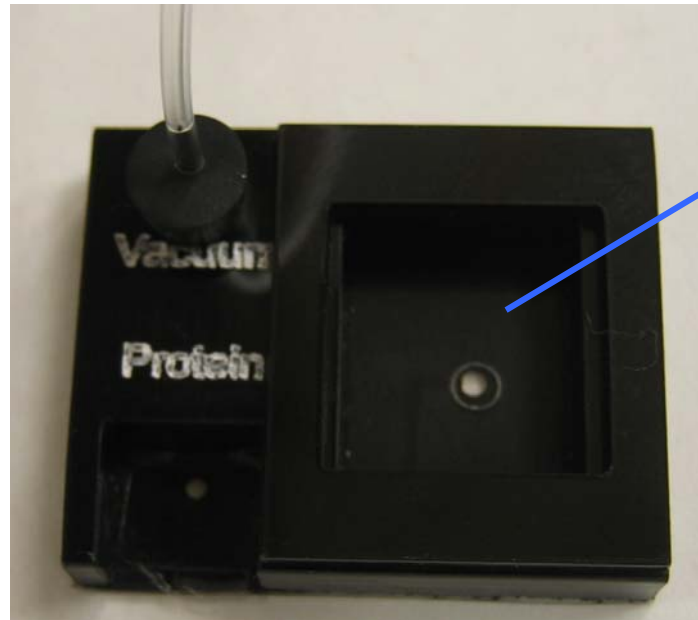
Vacuum tip: fit the 5mm dia. vacuum port of the XZ™ plates

3. Disposable needle (part# XZ01-02)



Fluoropolymer coated to prevent contamination

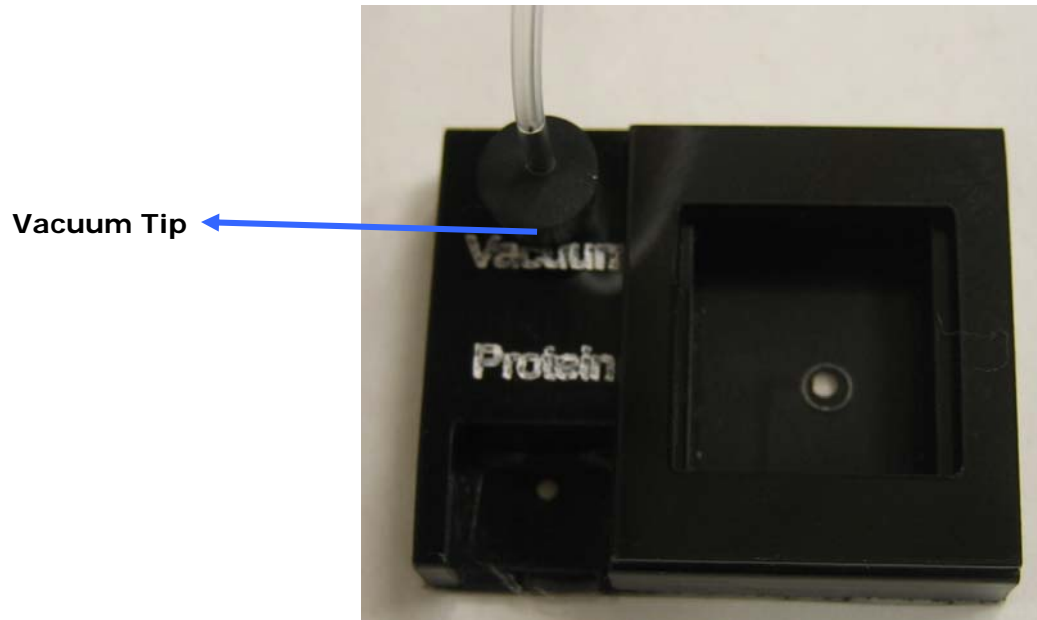
Step 1: Load Reagent



reagent well volume: 800µl

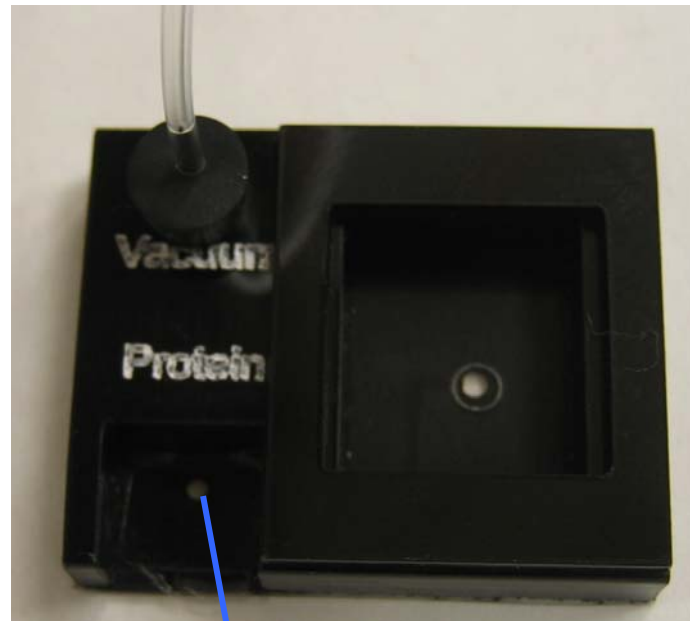
- Pipette 500µl of reagent into the reagent well.

Step 2: Set Up Vacuum Connection



- Turn on the vacuum source (vacuum pump, house vacuum, etc.)
- Connect the vacuum tubing adaptor to the vacuum source, and the vacuum tip to the vacuum port of the XZ™ plate. Make sure all connections are tight, **air bubbles in every dialysis chamber will result due to leaky connections.**
- **Start a timer to allow for 1 minute** evacuation of air from the dialysis chamber and channel in the XZ™ plate.

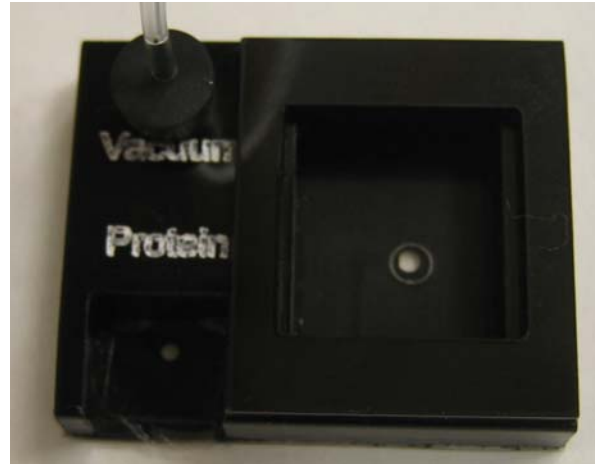
Step 3: Deposit Protein Sample Onto The Protein Inlet Film



protein inlet

- Pipette 1.5 μ l (4.5 μ l for 1x4 plates) of the protein sample onto the protein inlet film, make sure the protein droplet is bubble free and centered around the protein inlet.

Step 4: Load Protein Sample Into Dialysis Chambers

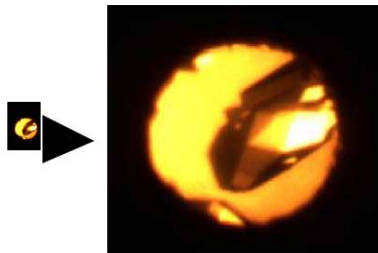


- **At the 1min mark of vacuum evacuation**, puncture the protein inlet film (through the sample solution) with a disposable needle (part# XZ01-02). The dialysis chamber will be filled with the protein sample due to the vacuum force. Maintain the vacuum on the XZ™ plate for another **30sec from the time the protein inlet film was punctuated**.
- Disconnect the vacuum tip from the XZ™ plate.
- Pipette 15µl of the purge & isolation liquid (XZ01-05) each at the protein inlet and in the vacuum port to seal off the microfluidic channel.
- Seal off the reagent well with a crystallization compatible clear film.
- End of sample and reagent loading.

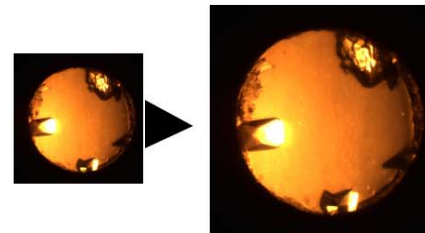
Step 5: Inspection of Experiments

- Inspect the dialysis chambers through a stereomicroscope use base/transmitted illumination. To minimize heat exposure that might cause vapor condensation on the sealing film, a cool light source for the microscope is recommended.

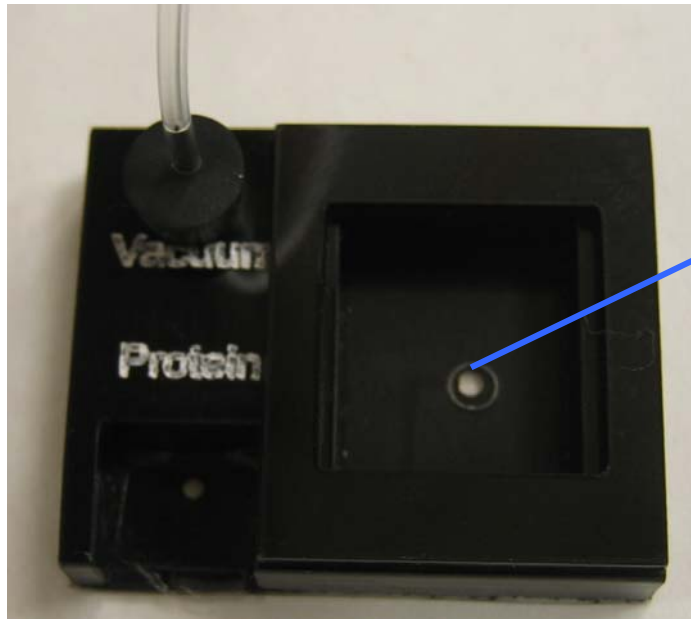
crystals grown in 240 μ m diameter dialysis chamber



crystals grown in 1mm diameter dialysis chamber



Step 6: Harvest Crystals



Use a pair of tweezers to gently remove the dialysis membrane disc to allow loop access to harvest crystals. The diameter of the dialysis chamber is 1.6mm