High Throughput 192 Well Microdrop Crystallization Using the Corning® Crystal*EX*™ 384 Well Protein Crystallization Microplates

Technical Bulletin

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Introduction

The wealth of information generated by genomics and proteomics has led to a need to translate this information into drug discovery and development. X-ray crystallography is one method to determine not only the structure of a protein, but also its possible ligands. Automated systems are now being developed for performing high throughput microvolume, sitting-drop vapor diffusion experiments. These systems usually include a liquid dispenser and a high throughput imaging system. The liquid dispenser must be capable of accurately and reproducibly dispensing nanoliter quantities of protein solution into the protein well. The high throughput imaging system must be capable of data analysis and data management.

To complement this high throughput system, we have used a newly developed microplate that utilizes the SBS footprint and has 384 well locations. The flat, hydrophobic and optically clear wells of the Corning Crystal*EX* 384 well microplates facilitate microdrop centering and allow shadow-free imaging of sitting-drop crystallization.

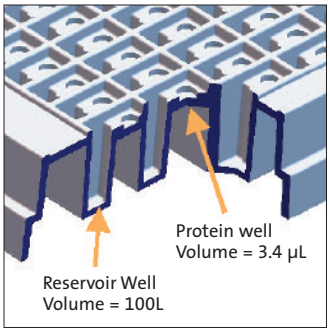

Figure 1. Crystal*EX* 384 Well Protein Crystallization Microplate is the only crystallization plate designed for full automation. The 192 reservoirs and corresponding protein wells are compatible with liquid dispensing heads.

These new Crystal*EX* 384 well protein crystallization microplates (Figure 1) were designed for use in high throughput crystallization (HTC) systems. Up to 192 separate sitting-drop vapor diffusion experiments can be performed in a single plate. Each plate contains 192 round reservoir wells, each with a single built-in protein well. The reservoir wells hold a working volume of 25 to 100 µL. In the sitting-drop format, crystallization occurs when vapor diffuses from the protein to the buffer well and the protein becomes supersaturated, forming a crystal.

The Crystal*EX* 384 well microplates are manufactured from an advanced proprietary polymer that reduces protein drop evaporation compared with polystyrene microplates. When protein and solvent are dispensed into the protein well, the drops do not separate, allowing for automated visualization. The polymer is highly resistant to commonly used crystallization solvents including methanol, DMSO, acetone and alcohols. The surface has high optical clarity and allows protein crystals to be easily viewed under polarized light. The reservoir and protein wells are positioned to be compatible with a variety of automated dispensing equipment.

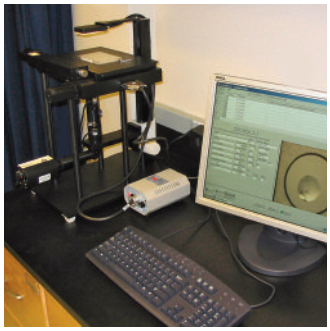

Figure 2. Cartesian Dispensing 16 +1 Honeybee Benchtop System includes 8 or 16 channels for mother liquor dispensing and an independent protein channel for protein dispensing.

The Cartesian™ Honeybee™ (Figure 2) is an automated liquid dispenser which features Synquad™ technology. This technology involves coupling of a highspeed microsolenoid value with a high-resolution syringe pump. In the aspirate and dispense mode, the mother liquor and protein solutions are taken up through the Synquad head and dispensed into the Crystal*EX* 384 well microplate. The Honeybee dispenser rapidly dispenses microvolume drops while fluidic inertia from the inkjet mechanism efficiently mixes the two solutions.

The CrystalScore™ system (Figure 3) is an automated imager that has a motorized x-y stage that automatically moves to each well and captures an image with a high-resolution digital camera (QI Imaging). The CrystalScore software saves each image to a relational database that also contains information about each experiment such as protein, reagents, concentration, pH and temperature. The software includes filtering tools that will automatically detect crystals in the drops and determine sizes and populations of crystals in a drop.

In this study, we demonstrate the use of the 384 well Crystal*EX* ™ plate as part of an automated high throughput crystallization system.

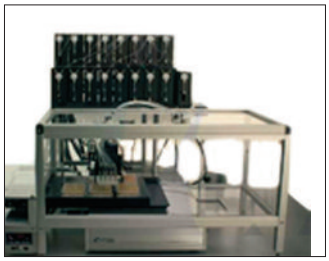

Figure 3. The CrystalScore system is an automated imager that has a motorized x-y stage that automatically moves to each well and captures an image with a high-resolution digital camera.

Materials and Methods

The Crystal*EX* 384 well protein crystallization microplates (Corning Cat. No. 3775) were obtained from Corning Life Sciences (Acton, MA). The Cartesian™ Honeybee™ Benchtop System from Genomic Solutions Inc. (Ann Arbor, MI) uses 16 Synquad channels for mother liquor dispensing and one independent protein solution dispensing channel. Included on the deck are six plate positions, a protein solution vial chiller, vacuum pump, air compressor and computer controller. The dispensing unit in enclosed in a humidity chamber that maintains 95 to 98% humidity. The CrystalScore imaging system is manufactured by Diversified Scientific, Inc. (Birmingham, AL). This instrument was modified so that it used inverted, bottom viewing optics instead of top-viewing optics. Crystallization solutions were purchased from Hampton Research, (Aliso Viejo, CA). Universal Optical sealing tape (Corning Cat. No. 6575) was used to seal the plates.

Protein Crystallization

Crystals of α-chymotrypsinogen and catalase were obtained by the sitting-drop method of vapor diffusion. Protein solutions of α-chymoptrypsinogen and catalase were dispensed into the protein wells of a Crystal*EX* 384 well microplate. Mother liquor was added to the protein well to make a final volume of 1 µL. The mother liquor was dispensed into the buffer reservoir well at a volume of 50 µL. The microplates were then sealed and incubated until visualization.

Liquid Dispensing

The Cartesian Honeybee was used to set up all crystallization screens. The screens are loaded into the 384 well Crystal*EX* microplates and the source solutions are loaded into the source plates. The microplates are placed in the appropriate locations on the instrument deck. The protein solution is contained in a microcentrifuge tube on the deck. The system then aspirates and dispenses these solutions using 16 tips for the screening solutions and one tip for the protein solution. The system washes the 16 tips and aspirates new solutions until set-up is complete for the entire plate.

The protein solutions are mixed 1:1 with mother liquor. This mixed solution is then equilibrated against a buffer reservoir containing the crystallization solution. The plates are sealed manually using optically clear sealing tape.

The total time for setting up 192 experimental screens in one plate is approximately fifteen minutes. The bulk of the time is for the repetitive solution handling that includes aspiration, dispensing and tip washing. The individual protein loading takes approximately one minute.

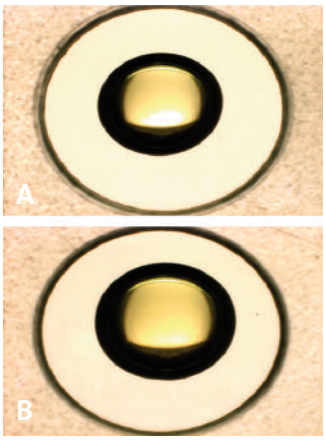

Figure 4. Water droplets dispensed multiple times into the center of the protein well. Three 200 nL drops were dispensed to create a 600 nL drop (A) and four 200nL drops were dispensed to yield an 800 nL drop (B).

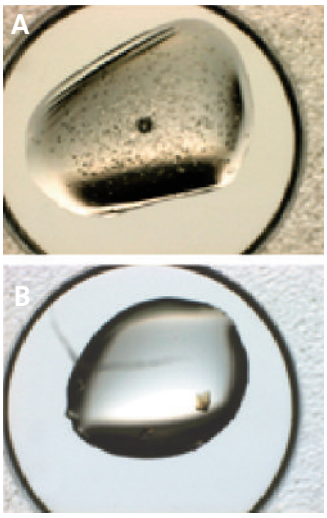

Figure 5. Crystals generated using Crystal*EX* 384 microplates and the Cartesian Honeybee dispenser. 200 nL drops of protein and mother liquor were dispensed multiple times to create a 1L drop. The crystals formed are - chymotrypsinogen (A) and catalase (B).

Evaporation is prevented by the humidity chamber. The final volume after several days is approximately one half of the starting volume. The microplate is then sealed and transferred to the incubator.

Imaging

The robotic system automatically transfers the microplates from the incubators to the CrystalScore™ system, collects the images, and moves the microplates back to the incubators. A scheduler feature in the software communicates to the robotic system how often and when to image each microplate.

Results

The Crystal*EX*™ 384 well microplate is a versatile tool that facilitates high throughput crystallography screening. The novel hydrophobic polymer and large flat bottom protein well prevents mother liquor and protein drop separation. This allows a 1 µL drop to stay centered so that it will not contact the sidewall of the protein well. The optically clear flat surface allows crystals to be easily viewed with minimal interference.

There is no separation of drops dispensed into the wells and no contact of the drops with the well sidewall (Figure 4). The drops in Figure 4 were created by dispensing 200 nL of water using the Cartesian™ Honeybee™ dispenser. Three 200 nL drops were dispensed to create a 600 nL drop and four 200 nL drops were dispensed to create an 800 nL drop. These submicroliter drops were used for testing the experimental set-up only, and were not used for actual crystallization experiments. All crystallization experiments described in this article were performed using a combined drop size of 1 µL or larger.

Crystals of α-chymotrypsinogen and catalase were grown in Crystal*EX* 384 well microplates with reagents dispensed from the Honeybee dispenser (Figure 5). Two hundred nanoliter drops of protein solution and mother liquor were dispensed multiple times to create a 1 µL drop. The crystals are easily imaged in the optically clear Crystal*EX* 384 well microplate using the CrystalScore imager.

Conclusions

The Crystal*EX* 384 well microplate, together with the Cartesian Honeybee nanoliter dispenser and CrystalScore imager, enables the rapid screening and production of 192 crystallization conditions per plate at a rate of approximately 800 conditions per hour. Throughput could be significantly increased by optimizing operational protocols or utilizing a dispensing system with additional dispensing tips. Additional capabilities include precise pipetting of the protein solution and mother liquor drop into the center of the protein well, which facilitates high throughput crystal imaging. The new Crystal*EX* 384 well microplate is a flexible tool which significantly reduces protein and reagent consumption, time and cost.

The Crystal*EX* 384 well microplate easily lends itself to the successful set-up of 192 separate experiments on custom-built and commercially available liquid handling platforms. The Crystal*EX* 384 well microplate eliminates bottlenecks by allowing multiple crystallization experiments to be performed and visualized on automated liquid handling and visualization systems.