Microlytic



# Crystal Former User Manual

## Crystal Former

A series of novel devices that harness the power of diffusive mixing to increase the likelihood of protein crystallization.

Catalogue Numbers: CF-O-20, CF-XL-20, CF-HT2-10

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## Original 16-channel Crystal Former

1. Unpack and label the Crystal Former devices. The 16-channel formats (CF and CF-XL) can be used with either the SH-1 or SH-2 slide holder.
2. Apply 0.5-1.0 µL of protein sample to the inlet on one side of the microfluidic channel.
3. Observe that the channel is completely filled via capillary action.
4. If using a different volume of precipitant, apply a single piece of sealing tape (RT-2) over the protein inlet by laying the tape strip gently on the inlet and smoothing the tape using a plastic card or equivalent. **Be careful not to apply too much pressure, as this will cause bubbles and gradient dissipation.** If using the same volume of precipitant, skip this step and perform sealing of both sets of inlets at the end.
5. Apply 0.5-1.0 µL of precipitant solution to the opposing inlet.
6. Seal the all channels as described in step 4.
7. Incubate the Crystal Formers on a flat surface. Incubation in a humidity cabinet prolongs the lifetime of the experiment.

## Scale-Up 16-channel Crystal Former

1. Unpack and label the Crystal Former devices. The 16-channel formats (CF and CF-XL) can be used with either the SH-1 or SH-2 slide holder.
2. Apply 1.5 µL of protein sample to the inlet on one side of the microfluidic channel.
3. Observe that the channel is completely filled via capillary action.
4. If using a different volume of precipitant, apply a single piece of sealing tape (RT-2) over the protein inlet by laying the tape strip gently on the inlet and smoothing the tape using a plastic card or equivalent. **Be careful not to apply too much pressure, as this will cause bubbles and gradient dissipation.** If using the same volume of precipitant, skip this step and perform sealing of both sets of inlets at the end.
5. Apply 0.5-1.5 µL of precipitant solution to the opposing inlet.
6. Seal the all channels as described in step 4.
7. Incubate the Crystal Formers on a flat surface. Incubation in a humidity cabinet prolongs the lifetime of the experiment.

## High Throughput Crystal Former

1. Unpack and label the Crystal Former devices.
2. Apply 0.3-1.0 µL of protein sample to the conical inlet on the right side of the microfluidic channel. Volumes as low as 0.15 uL may be used with robotics.
3. Observe that the channel is completely filled via capillary action.
4. Apply 0.5-1.0 µL of precipitant solution to the opposing (left) inlet.
5. Seal the entire plate with a pre-cut sealing film (MLY-VD-268) so as to minimize bubbles between the plate and sealing film. Be careful not to apply too much pressure, as this will cause bubbles to form within the channels and gradient dissipation.
6. Incubate the Crystal Formers on a flat surface. Incubation in a humidity cabinet prolongs the lifetime of the experiment.

| Crystallization Screens | | | |
|---|---|---|---|
| Cat. No | Product Name | Description | List Price (USD) |
| MLY-1 | PurePEGs | 48 conditions of PurePEGs | $125 |
| MLY-1-M | PurePEGs for Mosquito | 48 conditions of PurePEGs | $125 |
| MSS-1 | SuperSMART | 48 conditions of SuperSMART | $125 |
| MSS-1-M | SuperSMART for Mosquito | 48 conditions of SuperSMART | $125 |
| SSC-1 | SuperCOMBI | SuperSMART and PurePEGs | $195 |
| SSC-1-M | SuperCOMBI for Mosquito | SuperSMART and PurePEGs | $195 |

| Crystal Formers | | | |
|---|---|---|---|
| Cat. No | Product Name | Description | List Price (USD) |
| CF-O-20 | Original Crystal Former | 20 x 16-Channel Crystal Formers | $600 |
| CF-XL-20 | Scale-Up Crystal Formers | 20 x Scale-up Crystal Formers | $600 |
| CF-HT2-10 | SBS Crystal Former HT | 10 x SBS High Throughput Crystal Formers | $1,150 |

| Kits | | | |
|---|---|---|---|
| Cat. No | Product Name | Description | List Price (USD) |
| MCK2-HT | SBS Crystallization Kit | 10 x CF-HT2 , SuperCOMBI, Lysozyme Positive Control Kit, Sealing Film, X-acto Knife | $1,250 |
| MCK2-HT-M | SBS Crystallization Kit for Mosquito | 10 x CF-HT2, SuperCOMBI, Lysozyme Positive Control Kit, Sealing Film, X-acto Knife | $1,250 |

| Accessories | | | |
|---|---|---|---|
| Cat. No | Product Name | Description | List Price (USD) |
| RT-2 | Sealing Tape Strips | 200/roll for sealing Original and Scale-Up CFs | $25 |
| MLY-VD-268 | Optical Clear Film | 100 films for sealing CF-HT2 | $89 |
| MLY-VD-269 | Optical Clear Film-Value Pack | 1000 films for sealing CF-HT2 | $640 |
| MLY-Brayer | Sealing roller | Rubber roller for even sealing film application on microtiter plates | $25 |
| MLY-X-acto | Retractable X-acto Knife | | $12 |
| SH-1 | Snap-Fit Holder | for 16-channel original and scale-up chips | $100 |
| SH-2 | Drop-In Holder | for 16-channel original and scale-up chips | $100 |
| CF-MM | Magnetic Mount | for 16-channel original and scale-up chips | $25 |
| MiTeGen-Small | MiTeGen Crystal Harvesting Kit - Small | MicroLoop assortment, 1 x X-acto Knife, 1 x Tweezer, 5 x B3S Reusable Bases, 1 x Base Holder | $156.50 |
| MiTeGen-Large | MiTeGen Crystal Harvesting Kit - Large | MicroLoop assortment, 1 x X-acto Knife, 1 x Tweezer, 20 x B3S Reusable Bases, 1 x Base Holder | $339.50 |

The Crystal Former has been designed to enable gentle diffusive mixing of protein and precipitant within rigid microchannels. The Crystal Former's special materials, unique surface treatment and proprietary mixing kinetics thus combine to increase both the likelihood of crystallization and the quality of the resultant protein crystals.

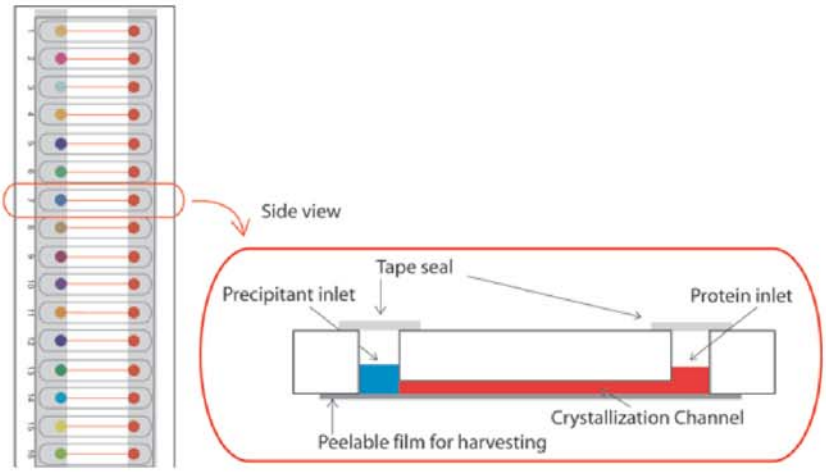
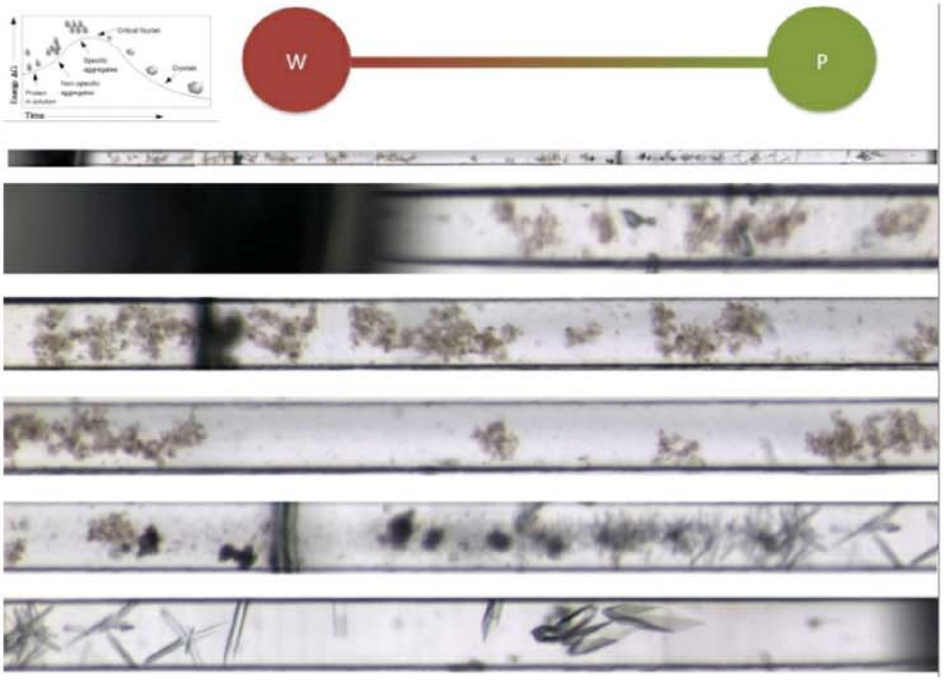



**Figure 1:** A cross sectional view of the original Crystal Former demonstrating complete channel filling by the protein solution and the formation of the protein-precipitant interface formed at the opening of the microfluidic channel

The Crystal Former is best described as a high output technique in which the number of crystallization events is relative large when compared to conventional protein crystallization experiments. The new SBS Crystal Former extends the data output even more so by coupling the high throughput of the original Crystal Former with a fully automatable, high throughput design.

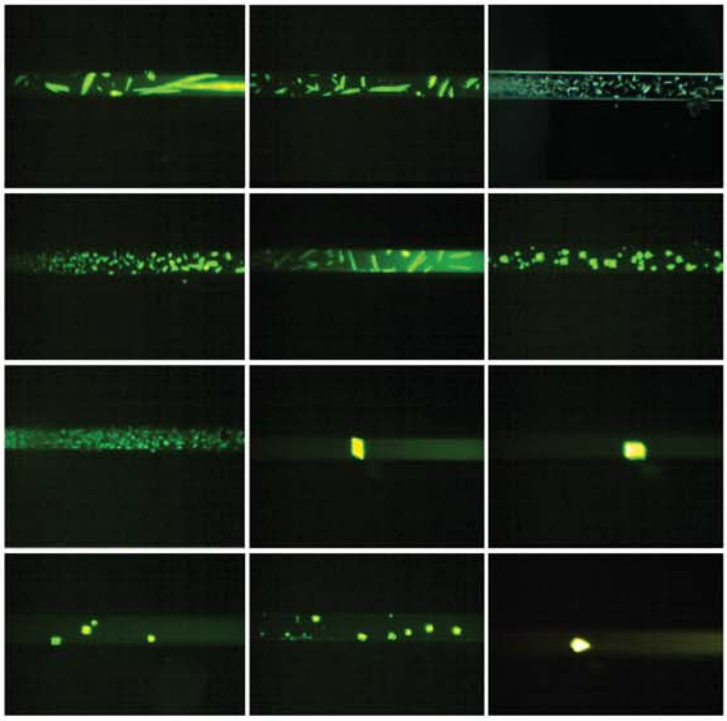



## Practical Advantages from the Crystal Former System

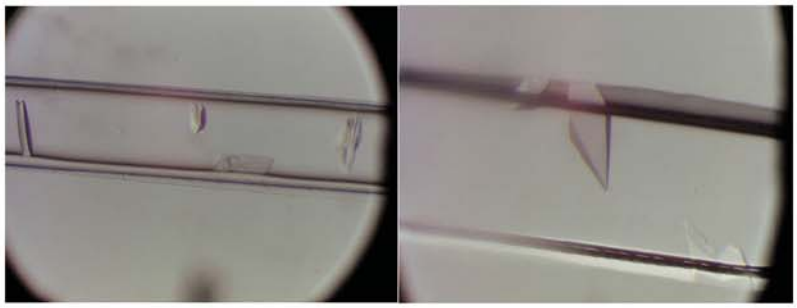
- Significantly increased likelihood of determining crystallization conditions
- Fewer discrete crystallization conditions need be explored, reducing the amount of protein necessary and maximizing the amount of protein behavior information obtained
- Each capillary provides a complete overview of protein behavior across the complete condition gradient
- The wealth of information about protein behavior guides follow-up experiments for optimization
- Crystals can be identified using fluorescent imaging of the Crystal Former

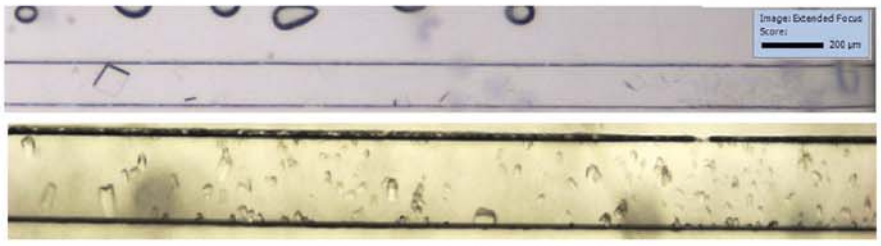
# Samples of crystals grown in the Crystal Former

Fluorescence imaging



Visible light imaging





## 1. Unpacking and labeling of the Crystal Former

Carefully remove the Crystal Former from its sealed aluminum wrapper and place it on a flat surface. Label the Crystal Former directly using a standard lab marker or by affixing a printed label or barcode to the Crystal Former slide holder (Part# SH-1 or SH-2).

## 2. Loading the Protein Solution

(a) To load the protein solution, first place the Crystal Former on a flat surface with the inlets placing up (see overview diagram).

*Tip: Placing a matte black surface under the Crystal Former will facilitate visualization of channel loading during set-up.*

(b) Aspirate the desired protein volume into the pipette tip. Typically, a protein volume of 0.5 µL is loaded into each inlet.

(c) Load protein to inlet. Gently lower the pipette tip into the first inlet. The tip should be lowered far enough for the liquid to enter the inlet when released. If pipetting by hand, one may hold the pipette at a 45-degree angle with the tip pointing toward the channel opening. The channel will fill via capillary action, during which the next inlet can be loaded.

*Tip: the protein inlets can be loaded simultaneously using an eight-channel pipette.*

## 3. Loading the Precipitant Solution

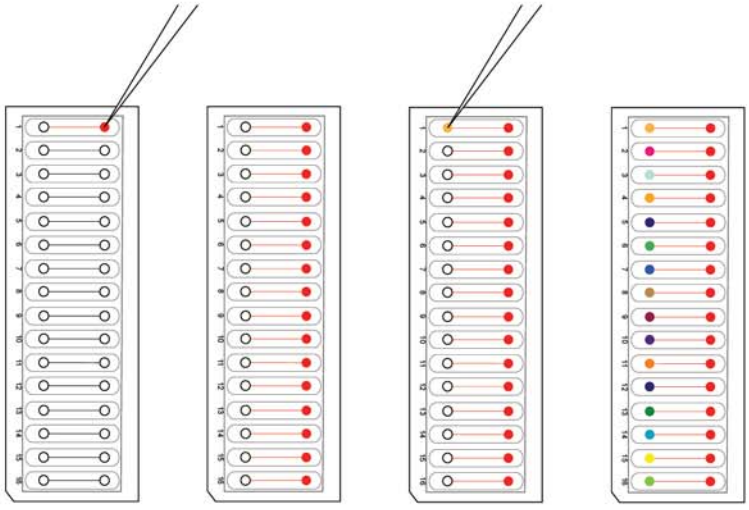
Precipitant solutions are loaded into the inlets opposite of the protein inlets to enable diffusive mixing in the microfluidic channel.

(a) Aspirate the desire volume of precipitant solution into the pipette tip. Typically, volumes of 0.5-1.0 µL are loaded into each inlet.

*Tip: By varying the ratio of protein volume to precipitant volume, different equilibration kinetics will be achieved.*
*Tip: to reproduce a particular result, it is critical to reload the same volumes as previously used in the initial experiment.*

(b) Load precipitant to opposite inlet. Gently lower the pipette tip into the first inlet. The tip does not need to touch the bottom of the inlet, but it should be close enough to the bottom so that when the drop is released, it contacts the protein solution at the outlet of the microfluidic channel. If pipetting by hand, on may hold the pipette at a 45-degree angle with respect to the surface of the table, pointing the tip away from the capillary opening, to avoid forceful mixing of solutions during ejection of solutions from pipette tip. At this point, the next channel can be loaded.

*Technical note: Fluidic contact forms between the precipitant solution in the inlet and the protein solution in the channel, enabling the diffusive mixing of the two solutions.*



## 4. Sealing the inlets

Use the supplied tape (Part #RT-2) to thoroughly seal inlets and reaction chambers. Using the supplied tape is recommended for keeping the center part of the Crystal Former clear for imaging. The sealing tape is a contact adhesive. Only slight pressure is needed to seal the inlets. Do not press down hard when sealing as this will lead to the introduction of bubbles in the channel and dissipation of the crystallization gradient.

## 5. Crystal Former storage and incubation

Once the Crystal Former is fully loaded and sealed, it can be stored resting on a flat surface or in a Crystal Former holder.

The Crystal Former can be stored at any standard temperature used in protein crystallography.

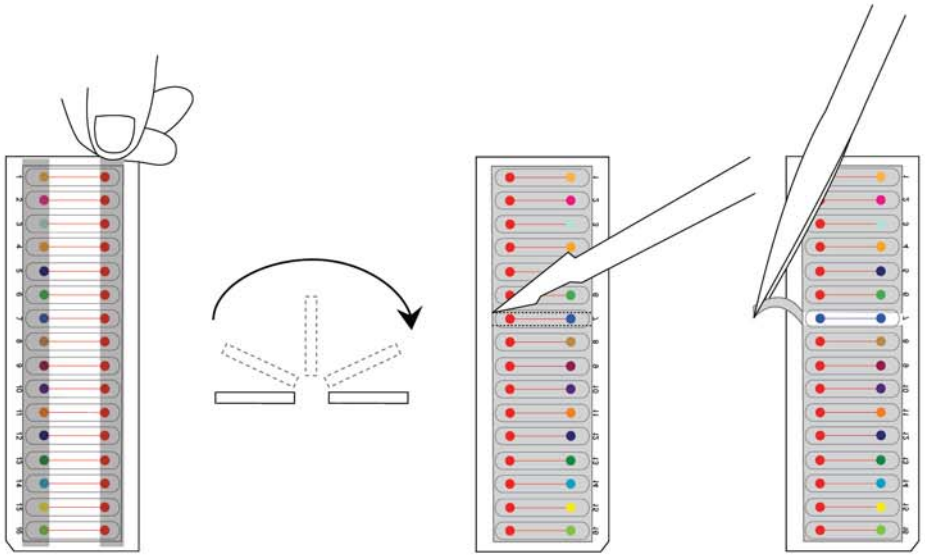
*Tip: The humidity of the area in which the Crystal Former is stored will have moderate effects on equilibration over the long term. If the Crystal Former is to be stored for longer than one week, it is recommended that users keep it in a humidified chamber.*

## 6. Harvesting Crystals

Before harvesting, prepare a cryoprotectant or stabilization solution. Turn over the Crystal Former and use a scalpel to cut the peelable film surrounding the reaction chamber where access is desired.
Once the film is completely cut, peel the film off using tweezers or fingers.

*Tip: Sometimes the crystal grows on the sealing film itself. It is recommended that the channel be opened under a microscope and the film inspected immediately for the presence of crystals. Crystals can be easily dislodged from the surface of the film, so these should not be discounted.*

Once the film is removed, a cryoprotectant or stabilizing solution should be applied immediately to both the open capillary and the cut film, to prevent dehydration while manipulating crystals inside chamber or on the film



## 1. Unpacking and labeling of the Crystal Former

Carefully remove the Crystal Former from its sealed aluminum wrapper and place it on a flat surface. Label the Crystal Former directly using a standard lab marker or by affixing a printed label or barcode to the Crystal Former slide holder (Part# SH-1 or SH-2).

## 2. Loading the Protein Solution

(a) To load the protein solution, first place the Crystal Former on a flat surface with the inlets placing up (see overview diagram).

*Tip: Placing a matte black surface under the Crystal Former will facilitate visualization of channel loading during set-up.*

(b) Aspirate the desired protein volume into the pipette tip. Typically, a protein volume of 1.5 µL is loaded into each inlet.

(c) Load protein to inlet. Gently lower the pipette tip into the first inlet. The tip should be lowered far enough for the liquid to enter the inlet when released. If pipetting by hand, one may hold the pipette at a 45-degree angle with the tip pointing toward the channel opening. The channel will fill via capillary action, during which the next inlet can be loaded.

*Tip: the protein inlets can be loaded simultaneously using an eight-channel pipette.*

## 3. Loading the Precipitant Solution

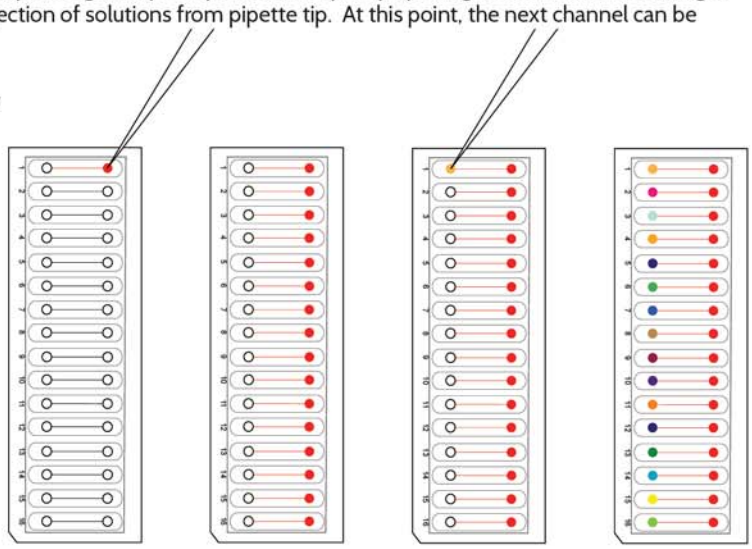
Precipitant solutions are loaded into the inlets opposite of the protein inlets to enable diffusive mixing in the microfluidic channel.

(a) Aspirate the desire volume, typically 0.5 - 1.5 µL, of precipitant solution into the pipette tip.

*Tip: By varying the ratio of protein volume to precipitant volume, different equilibration kinetics will be achieved.*
*Tip: to reproduce a particular result, it is critical to reload the same volumes as previously used in the initial experiment.*

(b) Load precipitant to opposite inlet. Gently lower the pipette tip into the first inlet. The tip does not need to touch the bottom of the inlet, but it should be close enough to the bottom so that when the drop is released, it contacts the protein solution at the outlet of the microfluidic channel. If pipetting by hand, on may hold pipette at a 45-degree angle with respect to the surface of the table, pointing the tip away from the capillary opening, to avoid forceful mixing of solutions during ejection of solutions from pipette tip. At this point, the next channel can be loaded.

*Technical note: Fluidic contact forms between the precipitant solution in the inlet and the protein solution in the channel, enabling the diffusive mixing of the two solutions.*



## 4. Sealing the inlets

Use the supplied tape (Part #RT-2) to thoroughly seal inlets and reaction chambers. Using the supplied tape is recommended for keeping the center part of the Crystal Former clear for imaging. The sealing tape is a contact adhesive. Only slight pressure is needed to seal the inlets. Do not press down hard when sealing as this will lead to the introduction of bubbles in the channel and dissipation of the crystallization gradient.

## 5. Crystal Former storage and incubation

Once the Crystal Former is fully loaded and sealed, it can be stored resting on a flat surface or in a Crystal Former holder.

The Crystal Former can be stored at any standard temperature used in protein crystallography.

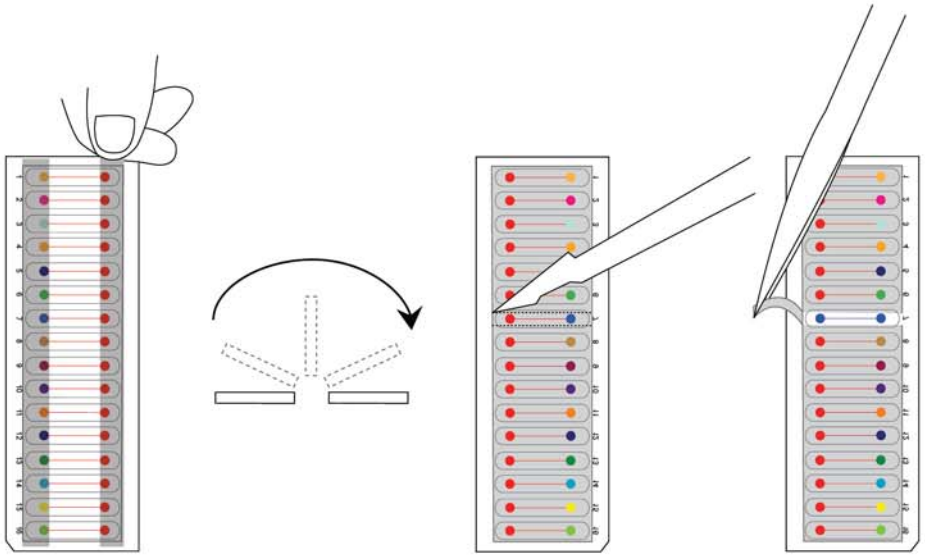
*Tip: The humidity of the area in which the Crystal Former is stored will have moderate effects on equilibration over the long term. If the Crystal Former is to be stored for longer than one week, it is recommended that users keep it in a humidified chamber.*

## 6. Harvesting Crystals

Before harvesting, prepare a cryoprotectant or stabilization solution. Turn over the Crystal Former and use a scalpel to cut the peelable film surrounding the reaction chamber where access is desired.
Once film is completely cut, peel the film off using tweezers or fingers.

*Tip: Sometimes the crystal grows on the sealing film itself. It is recommended that the channel be opened under a microscope and the film inspected immediately for the presence of crystals. Crystals can be easily dislodged from the surface of the film, so these should not be discounted.*

Once the film is removed, a cryoprotectant or stabilizing solution should be applied immediately to both the open capillary and the cut film, to prevent dehydration while manipulating crystals inside chamber or on the film.



## 1. Unpacking and labeling of the Crystal Former

Carefully remove the Crystal Former from its sealed aluminum wrapper and place it on a flat surface. Label the Crystal Former directly using a standard lab marker or by affixing a printed label or barcode to the Crystal Former plate skirt.

## 2. Loading the Protein Solution

a) To load the protein solution, first place the Crystal Former on a flat surface with the inlets facing up (see overview diagram).

*Tip: Placing a matte black surface under the Crystal Former will facilitate visualization of channel loading during set-up.*

(b) Aspirate the desired protein volume into the pipette tip. Typically, a protein volume of 0.3-0.5 µL is loaded into each inlet, but higher volumes of up to 1.0 uL may be used, if desired.

(c) Load protein to the funneled inlet on the right side of each channel. Gently lower the pipette tip into the first inlet. The tip should be lowered to the narrowed portion of the inlet. The channel will fill via capillary action, during which the next inlet can be loaded.

*Tip: the protein inlets can be loaded simultaneously using an eight-channel pipette.*

## 3. Loading the Precipitant Solution

Precipitant solutions are loaded into the cylindrical inlets on the left side of each of the channels to enable diffusive mixing in the microfluidic channel.

(a) Aspirate the desired volume, typically 0.5-1.0 uL, of precipitant solution into the pipette tip.

*Tip: By varying the ratio of protein volume to precipitant volume, different equilibration kinetics will be achieved.*

*Tip: to reproduce a particular result, it is critical to reload the same volumes as previously used in the initial experiment.*

(b) Load precipitant to inlet. Gently lower the pipette tip into the first inlet. The tip does not need to touch the bottom of the inlet, but it should be close enough to the bottom so that when the drop is released, it contacts the protein solution at the outlet of the microfluidic channel. At this point, the next channel can be loaded.

*Technical note: Fluidic contact forms between the precipitant solution in the inlet and the protein solution in the channel, enabling the diffusive mixing of the two solutions.*

### *4. Sealing the inlets*

Use the supplied optical clear sealing film tape (Part #MLY-VD-268) to thoroughly seal inlets and reaction chambers. The sealing tape is a contact adhesive. Only slight pressure is needed to seal the inlets. Do not press down hard when sealing as this will lead to the introduction of bubbles in the channel and dissipation of the crystallization gradient.

### *5. Crystal Former storage and incubation*

Once the Crystal Former is fully loaded and sealed, it can be stored resting on any flat surface.

The Crystal Former can be stored at any standard temperature used in protein crystallography.

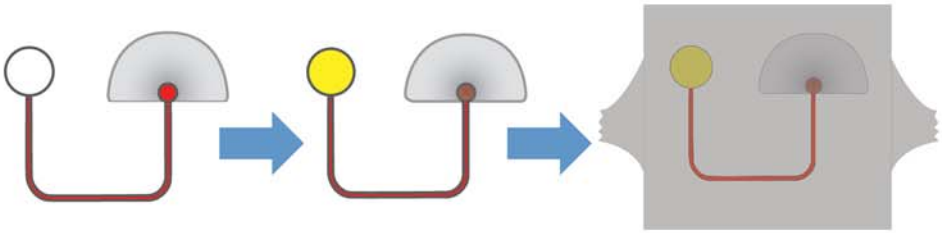
*Tip: The humidity of the area in which the Crystal Former is stored will have moderate effects on equilibration over the long term. If the Crystal Former is to be stored for longer than one week, it is recommended that users keep it in a humidified chamber.*

### *6. Harvesting Crystals*

Before harvesting, prepare a cryoprotectant or stabilization solution. Turn over the Crystal Former and use a scalpel to cut the peelable film surrounding the reaction chamber where access is desired and remove the entire sealing film square covering particular capillary and corresponding inlets. To do this, use the scalpel to cut the two or three tabs connecting that square to the neighboring seals. Once the tabs are completely cut, peel the film off using tweezers or fingers.

*Tip: Sometimes the crystal grows on the sealing film itself. It is recommended that the channel be opened under a microscope and the film inspected immediately for the presence of crystals. Crystals can be easily dislodged from the surface of the film, so these should not be discounted.*

Once the film is removed, a cryoprotectant or stabilizing solution should be applied immediately to both the open capillary and the cut film, to prevent dehydration while manipulating crystals inside chamber or on the film.



## Lysozyme Positive Control Kit

The kit contains:

(1) Solubilized Lysozyme at a final concentration of 100 mg/mL in 0.02 M Sodium Acetate Trihydrate pH 4.6
(2) Positive control crystallization condition: 1.2 M Sodium Chloride, 0.1 M Tri-sodium Citrate pH 3.5

Dispense the appropriate volume of Lysozyme into the protein inlet of any Crystal Former format. Allow the channel to fill completely with protein. Add same volume of crystallization solution to the opposite precipitant inlet. You should observe crystals within 24 hours that can be used for crystal harvesting practice.

## Automated Set-up of the High Throughput Crystal Former

The new SBS High throughput Crystal Former is compatible with a variety of robotics for both crystallization and imaging. Please visit our website (www.microlytic.com) or contact support@microlytic.com for additional information about these and other systems.

Art Robbins Instruments
www.artrobbins.com
www.artrobbins.com/contact-us/

Douglas Instruments
www.douglas.co.uk
info@douglas.co.uk

Formulatrix
http://www.formulatrix.com/
support@formulatrix.com

Labcyte Inc.
www.labcyte.com
http://www.labcyte.com/Contact_Us/Default.25.html

Rigaku Automation
www.rigaku.com
support@rigaku.com

TTP LabTech
www.ttplabtech.com

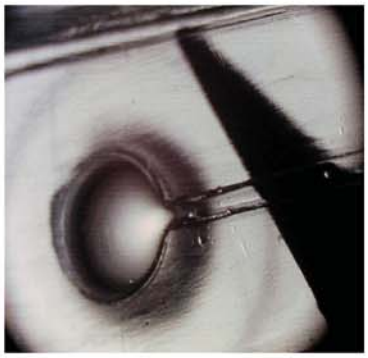
## 1. Crystals are harvested through removal of the rear sealing film (i.e. the side of the chip NOT containing sample inlets)

CF-HT from front
(sample inlets are closest to the front).

## 2. With a sharp edge, score the rear sealing film on either side of the microchannel.

Score the film 2-3 times to ensure that it has been cut completely. Incomplete cutting will disturb the neighboring channels as the sealing film is lifted.

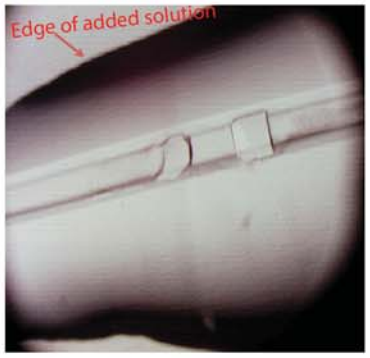
Lift the sealing film away from one end of the channel using the tip of the scalpel while inspecting the removal under the microscope to ensure that the crystal remains in the microchannel and is not attached to the sealing film.



## 3. Apply 5-10µL of stabilization solution or cryoprotectant to the opened microchannel

The large surface area of the microchannal will result in rapid dehydration of the crystallization experiment without the addition of solution.

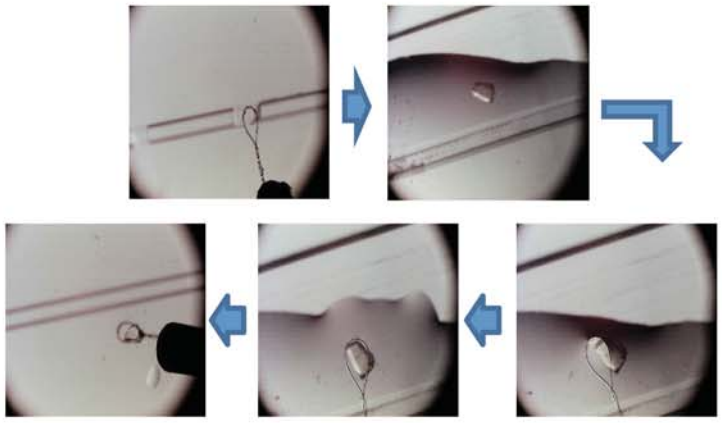
This step may use a preselected cryoprotectant, the crystallization condition, 50% (v/v) crystallization condition, mineral or paraffin oil, etc. As the channel is likely to be fully equilibrated at harvesting, you may consider all steps from 2 onward to be similar to harvesting from sitting drop.



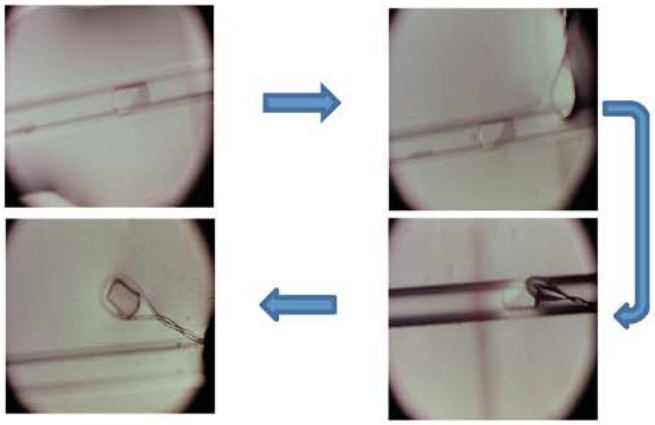

## 4. The crystal may be approached directly using a sample loop or crystal mounting tool of your choice.

Smaller loops are easily accommodated within the channel. Larger loops may also be used through deformation of the loop during insertion in the channel. The tips of larger loops may also be used to gently prod the crystal out of the channel and into the added solution.

4B. Crystals may also be dislodged from the channel with your preferred manipulation tool/loop for mounting outside of the channel



5. Scoop the crystal directly out of the channel using the loop and proceed with additional manipulations.



***-How do I harvest many crystals for further work without resealing the channel?***
We recommend that you move as many crystals as possible to a sitting drop stage with additional crystallization solution for longer-term storage during crystal mounting and testing and during optimization of cryoprotection conditions. This will permit you to store the crystals throughout the day and longer for prolonged soaking and diffraction experiments.

***-I have a single crystal and no idea about cryoprotection – what can I try?***
In this instance, an oil mount may be a good alternative. Apply 5-10 µL of paraffin, paratone or a combination of the two oils to the open microchannel. Loop the desired crystal and pull it through the oil layer with the loop perpendicular to the surface of the oil drop. Freeze the crystal immediately. The low volume of crystallization solution and the larger microchannel surface area will minimize the mother liquor that accompanies the crystal, especially when a loop that closely approximates the crystal size is used.

## *Three step procedure for evaluating the Crystal Former*

In order to evaluate the Crystal Former and compare it to your existing workflow, we suggest that you design a set of experiments for proteins that are:

- *Known to produce crystals under well defined conditions in the Crystal Former (Table 1)*
- *Previously crystallized using your current workflow*
- *Not crystallized using your current workflow*

### *(1) Model proteins known to produce crystals in Crystal Former*

To familiarize yourself with the features and format of the Crystal Former we suggest that you use one Crystal Former to grow crystals of a suitable model protein (e.g. Lysozyme, Xylanase or Thaumatin). This procedure will allow you to test your loading and sealing technique and to hone your crystal harvesting skills using a non precious protein sample.

Examples of suitable model proteins and their crystallization and cryo conditions are listed in Table 1. Simply load 0.5 µL of protein solution and 0.5 µL of precipitant solution as described in the user manual. To cryo protect crystals, add 5 µL of suggested cryo solution (Table 1) to the opened channel (see user manual for details).

***Table 1: Well characterized crystallization conditions for the Crystal Former***

| **Test protein** | **Hen egg white Lysozyme** | **Xylanase XYNII** | **Thaumatin** |
|---|---|---|---|
| Supplier | Sigma (A7641) | Hampton Research (HR7-104) | Sigma (T7638) |
| Protein buffer | 50 mg/mL in de-ionized water | Dilute to 18 mg/mL in de-ionized water | 50 mg/mL in de-ionized water |
| Precipitant | 20 % w/v Sodium chloride in 0.1 M Sodium acetate pH 5.5 | 0.2 M trimethylamine N-oxide, 0.1 M TRIS pH 8.5, 20 % w/v PEG 2000mme | 0.8 M Potassium sodium tartrate, 0.1 M HEPES pH 7.5 (Crystal Screen 1 #29) |
| Cryo solution | 25% glycerol, 10 % w/v Sodium chloride in 0.0.5 M Sodium acetate pH 5.5 | 25% glycerol, 0.1 M trimethylamine N-oxide, 0.05 M TRIS pH 8.5, 10 % w/v PEG 2000mme | 25% glycerol, 0.8 M Potassium sodium tartrate, 0.1 M HEPES pH 7.5 |
| Incubation time | 1-7 days | 3-7 days | 3-7 days |

### *(2) Proteins crystallized using current workflow*

We advise you to use the Crystal Former to re- screen a set of proteins, for which you were able to get crystal hits in your initial screening using your existing workflow.

For this part of evaluation it is important that you do not simply retest Crystal Former with the conditions known to give crystals in vapor diffusion. Rather you should re-screen the protein either using your standard sparse matrix screen that was used when screening using your current workflow or using a screen optimized for the Crystal Former such as the SuperSMART Screen or PurePEG. The rationale for this approach is that the diffusive mixing kinetics of the Crystal Former are fundamentally different from vapor diffusion or batch based methods, thus it is not guaranteed that all crystallization conditions giving crystals using batch or vapor diffusion will give crystals using the Crystal Former.

We recommend that you select one or two proteins, that have previously been crystallized using your existing workflow, and set up *de novo* crystallization trials using the Crystal Former and SuperSMART Screen or PurePEGs. From this evaluation you can determine whether you can get equivalent results using the Crystal Former as using your existing workflow.

### *(3) Proteins not crystallized using current workflow*

The true power of the Crystal Former is the improved crystallization hit rate, which may allow you to get crystals from proteins that you have previously not been able to crystallize. In your evaluation of the Crystal Former we encourage that you select several proteins that are biophysically well behaved, but difficult to crystallize using your current workflow. For these proteins we recommend setting up crystallization trials using the Crystal Former and one or more of the sparse matrix screens that have been optimized specifically for the Crystal Former (e.g. SuperSMART Screen and/or PurePEGs).

From this evaluation you will be able to test whether the unique properties of the Crystal Former can facilitate crystallization of even these difficult to crystallize proteins.

**NOTE ON PROTEIN CONCENTRATION**: Protein concentration in the loading buffer, usually water or dilute buffer, should be approximately 60% – 80% of saturation; at least 10 – 30 mg/mL.

# Crystallization Screens

*SuperSmart Sparse Matrix Screen for Diffusive Mixing*

Diffusive mixing offers tremendous advantages in protein crystallization, including the ability to assess protein phase behavior across the gradient of a given condition for each microchannel used. In contrast to vapor diffusion, exploration of crystallization space becomes more systematic and complete in this format. The Crystal Former is an innovative device that harnesses the power of diffusive mixing in a simple, automatable format which, when coupled with optimized crystallization reagents, offers excellent coverage of crystallization space in an efficient and cost-effective manner.

The SuperSmart screen has been designed to offer efficient crystallization screening without the redundancy present in sparse matrix screens designed for vapor diffusion methods. The SuperSmart collection of conditions draws on the extensive history of crytallization trials at the Midwest Center for Structural Genomics and represents the most productive crystllization conditions commercially available in a format that maximizes the potential of the Crystal Former.

The 48 conditions of the SuperSMART Screen are also available as part of the SuperCOMBI Screen, which offers 96 crystallization conditions for comprehensive and innovative screening using the Crystal Former.

| SuperSMART Screen - Ordering Information | | | |
|---|---|---|---|
| Cat. No | Product Name | Description | List Price (USD) |
| MSS-1 | SuperSMART | 48 conditions of SuperSMART | $125 |
| MSS-1-M | SuperSMART for Mosquito | 48 conditions of SuperSMART | $125 |

# SuperSMART Screen

| Screen # | MSS position | CombiSmart position | Salt | Buffer | Precipitant | Precipitant 2 |
|---|---|---|---|---|---|---|
| 1 | A1 | A1 | 0.1 M sodium chloride | 0.1 M Bis-Tris:HCl pH 6.5 | 1.5 M Ammonium Sulfate | |
| 2 | A2 | A2 | 0.2 M ammonium phosphate monobasic | 0.1 M Tris:HCl pH 8.5 | 50% (v/v) MPD | |
| 3 | B1 | A3 | 0.2 M calcium chloride | 0.1 M Bis-Tris:HCl pH 5.5 | 45% (v/v) MPD | |
| 4 | B2 | A4 | 0.2 M sodium chloride | 0.1 M $Na_2HPO_4/KH_2PO_4$ pH 6.2 | 20% (w/v) PEG 1000 | |
| 5 | C1 | A5 | | 0.1 M Bis-Tris:HCl pH 6.5 | 28 % (w/v)PEG 2000 MME | |
| 6 | C2 | A6 | 0.1 M potassium thiocyanate | | 30% (w/v) PEG 2000 MME | |
| 7 | D1 | A7 | 0.15 M potassium bromide | | 30% (w/v) PEG 2000 MME | |
| 8 | D2 | A8 | | | 25% (w/v) PEG 2000 MME | |
| 9 | E1 | A9 | 0.1 M sodium acetate | 0.1 M MES:NaOH pH 6.5 | 30% (w/v) PEG 2000 MME | |
| 10 | E2 | A10 | | 0.1 M $Na_2HPO_4$:Citric acid pH 4.2 | 40% (v/v) PEG 300 | |
| 11 | F1 | A11 | 0.2 M calcium acetate hydrate | 0.1 M sodium cacodylate:HCl pH 6.5 | 40% (v/v) PEG 300 | |
| 12 | F2 | A12 | 0.2 M ammonium sulfate | 0.1 M Bis-Tris:HCl pH 5.5 | 25% (w/v) PEG 3350 | |
| 13 | G1 | B1 | 0.2 M magnesium chloride | 0.1 M HEPES:NaOH pH 7.5 | 25% (w/v) PEG 3350 | |
| 14 | G2 | B2 | 0.2 M sodium chloride | 0.1 M Bis-Tris:HCl pH 5.5 | 25% (w/v) PEG 3350 | |
| 15 | H1 | B3 | 0.2 M ammonium acetate | 0.1 M Bis-Tris:HCl pH 5.5 | 25% (w/v) PEG 3350 | |
| 16 | H2 | B4 | 0.2 M magnesium chloride | 0.1 M Bis-Tris:HCl pH 6.5 | 25% (w/v) PEG 3350 | |
| 17 | A6 | B5 | 0.2 M magnesium chloride | 0.1 M Tris:HCl pH 8.5 | 25% (w/v) PEG 3350 | |
| 18 | A7 | B6 | 0.2 M sodium thiocyanate pH 6.9 | | 20% (w/v) PEG 3350 | |
| 19 | B6 | B7 | 0.2 M potassium thiocyanate | | 20% (w/v) PEG 3350 | |
| 20 | B7 | B8 | 0.2 M potassium sodium tartrate | | 20% (w/v) PEG 3350 | |
| 21 | C6 | B9 | | 0.1 M Tris:HCl pH 8.5 | 25% (w/v) PEG 3350 | |
| 22 | C7 | B10 | 0.2 M potassium iodide | | 20% (w/v) PEG 3350 | |
| 23 | D6 | B11 | 0.2 M lithium acetate | | 20% (w/v) PEG 3350 | |
| 24 | D7 | B12 | 0.2 M potassium chloride | | 20% (w/v) PEG 3350 | |
| 25 | E6 | C1 | 0.2 M lithium sulfate | 0.1 M HEPES:NaOH pH 7.5 | 20% (w/v) PEG 3350 | |
| 26 | E7 | C2 | 0.1 M magnesium chloride | 0.1 M MES:NaOH pH 6.5 | 30% (v/v) PEG 400 | |
| 27 | F6 | C3 | | 0.1 M CAPS:NaOH pH 10.5 | 30% (v/v) PEG 400 | |
| 28 | F7 | C4 | 0.17 M ammonium acetate | 0.085 M sodium citrate:HCl pH 5.6 | 25.5% (w/v) PEG 4000 | 15% (v/v) glycerol |
| 29 | G6 | C5 | 0.17 M sodium acetate | 0.085 M Tris:HCl pH 8.5 | 25.5% (w/v) PEG 4000 | 15% (v/v) glycerol |
| 30 | G7 | C6 | 0.16 M magnesium chloride | 0.08 M Tris:HCl pH 8.5 | 24% (w/v) PEG 4000 | 20% (v/v)glycerol |
| 31 | H6 | C7 | 0.2 M calcium chloride | 0.1 M Tris:HCl pH 8.5 | 25% (w/v) PEG 4000 | |
| 32 | H7 | C8 | 0.2 M ammonium sulfate | 0.1 M sodium citrate:HCl pH 5.6 | 25% (w/v) PEG 4000 | |
| 33 | A11 | C9 | 0.8 M lithium chloride | 0.1 M Tris:HCl pH 8.5 | 32% (w/v) PEG 4000 | |
| 34 | A12 | C10 | | 0.1 M Bis-Tris:HCl pH 6.5 | 20% (w/v) PEG 5000 MME | |
| 35 | B11 | C11 | 0.2 M ammonium sulfate | 0.1 M MES:NaOH pH 6.5 | 30% (w/v) PEG 5000 MME | |
| 36 | B12 | C12 | 0.05 M calcium chloride | 0.1 M Bis-Tris:HCl pH 6.5 | 30% (v/v) PEG 550 MME | |
| 37 | C11 | D1 | 0.05 M magnesium chloride | 0.1 M HEPES:NaOH pH 7.5 | 30% (v/v) PEG 550 MME | |
| 38 | C12 | D2 | | 0.1 M sodium citrate:Citric acid pH 5.5 | 40% (v/v) PEG 600 | |
| 39 | D11 | D3 | 1 M lithium chloride | | 30% (w/v) PEG 6000 | 0.1 M sodium acetate |
| 40 | D12 | D4 | | 0.1 M HEPES:NaOH pH 7.5 | 20% (w/w) PEG 8000 | |
| 41 | E11 | D5 | 0.2 M calcium acetate hydrate | 0.1 M MES:NaOH pH 6.0 | 20% (w/v) PEG 8000 | |
| 42 | E12 | D6 | 0.2 M magnesium chloride | 0.1 M Tris:HCl pH 8.5 | 20% (w/v) PEG 8000 | |
| 43 | F11 | D7 | 0.2 M sodium chloride | 0.1 M $Na_2HPO_4$:Citric acid pH 4.2 | 20% (w/v) PEG 8000 | |
| 44 | F12 | D8 | 0.2 M ammonium sulfate | 0.1 M sodium cacodylate:HCl pH 6.5 | 30% (w/v) PEG 8000 | |
| 45 | G11 | D9 | 0.05 M ammonium sulfate | 0.05 M Bis-Tris:HCl pH 6.5 | 30% (v/v) pentaerythritol ethoxylate (15/4_EO/OH) | |
| 46 | G12 | D10 | 0.2 M calcium chloride | 0.1 M HEPES:NaOH pH 7.5 | 28% (v/v) PPG P400 | |
| 47 | H11 | D11 | 0.2 M magnesium chloride | 0.1 M HEPES:NaOH pH 7.5 | 30% (v/v) PPG P400 | |
| 48 | H12 | D12 | 0.2 M magnesium chloride | 0.1 M Tris:HCl pH 7 | 2.5 M sodium chloride | |

The PurePEGs screen features conditions with a mixture of purified PEGs, ranging from 300 MW to 8,000 MW. At a total PEG concentration of 22.5% combined with various salts and buffers, the conditions are designed to give the most crystallization hits spanning a wide pH range (1.1 - 9.8).

**Features:**

- Ultra pure PEGs - USP grade
- Optimized for diffusive mixing; capillary crystallization
- Polydisperse PEGs; 5 PEGs in each condition
- pH measured and recorded for final solution
- Better control of manufacturing impurities in the PEGs

Many successful crystallization conditions are a combination of polyethyleneglycols (PEGs) and salts adjusted to a particular pH. However, in an initial sparse screen, sampling the vast space of different salts versus different molecular weight (MW) PEGs, at a range of pH's, is challenging. Interestingly, there is evidence to suggest that protein crystallization hits obtained using a particular PEG can be repeated when using a mixture of PEGs. Consequently, a more rational screening strategy should use mixtures of PEGs rather than individual conditions. Microlytic has now developed such a PEG cocktail containing PEGs ranging from 300 – 8,000 MW.

Additionally, the pH of various sparse screen solutions has been observed to significantly differ from what can be inferred from the precipitant composition. A common misconception is that the presence of a buffer adjusted to a particular pH, using HCl or NaOH, will have sufficient buffering capacity to overcome the acidic or basic nature of the other components of the condition. Since most crystallization screens are manufactured simply by adding individual pre-formulated components to generate a final solution, without adjusting or even measuring the pH of the final solution, there are bound to be significant discrepancies between the expected and actual pH of the precipitant condition. Particularly for conditions containing technical grade PEGs, variable concentrations of sodium sulfate, sodium hydroxide or sulfuric acid, will affect the pH and the salts in the final solution. Anecdotally, there has been an ongoing issue with lithium sulfate and PEG solutions producing crystals of salts; we believe that the precipitation is due to residual sodium sulfate from the manufacturing process causing super saturation of salts in mixture. As a consequence, protein crystallographers trying to optimize a crystallization hit from such a condition may have an incorrect starting point for their efforts.

To address these issues Microlytic is introducing a new screen containing a new series of PEGs that are USP grade, highly purified to avoid unwanted contaminants and improve reproducibility of experments. Further, Microlytic lists the final pH of the crystallization conditions rather than the pH of the concentrated buffer components to provide protein crystallographers with as precise a

The screen is available in two storage formats. MLS-1 uses a Kisker full-skirted PCR plate with 8 cap-strips providing an easy resealing method for long term storage and repetitive usage. This format is NOT compatible with the Mosquito robot. MLS-1M uses a U-bottom Greiner assay plate with heat seal specially selected for Mosquito compatibility. MLS-1M accompanies the MCK-HT-M kit.

PurePEGs Screen - Ordering Information

| Cat. No | Product Name | Description | List Price (USD) |
|---|---|---|---|
| MLY-1 | PurePEGs | 48 conditions of PurePEGs | $125 |
| MLY-1-M | PurePEGs for Mosquito | 48 conditions of PurePEGs | $125 |

# PurePEGs Screen

| MLY# | Position | Condition | Final pH |
|---|---|---|---|
| 1 | A1 | 0.3 M Ammonium chloride, 0.1 M Citric acid, 22.5% PurePEGs PEG 0.3-8 kD | 4 |
| 2 | A2 | 0.3 M Ammonium formate, 0.1 M Potassium nitrate, 22.5% PurePEGs PEG 0.3-8 kD | 6.6 |
| 3 | B1 | 0.3 M Ammonium nitrate, 0.1 M Sodium citrate tribasic dihydrate, 22.5% PurePEGs PEG 0.3-8 kD | 5.9 |
| 4 | B2 | 0.3 M Ammonium phosphate monobasic, 0.1 M MES monohydrate, 22.5% PurePEGs PEG 0.3-8 kD | 5.7 |
| 5 | C1 | 0.3 M Ammonium sulfate, 0.1 M BIS-TRIS, 22.5% PurePEGs PEG 0.3-8 kD | 6.8 |
| 6 | C2 | 0.3 M Di-ammonium tartrate, 0.1 M Sodium acetate trihydrate, 22.5% PurePEGs PEG 0.3-8 kD | 5.8 |
| 7 | D1 | 0.3 M Cadmium chloride, 0.1 M Sodium acetate trihydrate, 22.5% PurePEGs PEG 0.3-8 kD | 3.7 |
| 8 | D2 | 0.3 M Cadmium sulfate, 0.1 M Imidazole, 22.5% PurePEGs PEG 0.3-8 kD | 4.6 |
| 9 | E1 | 0.3 M Caesium chloride, 0.1 M HEPES , 22.5% PurePEGs PEG 0.3-8 kD | 7.6 |
| 10 | E2 | 0.3 M Calcium acetate, 0.1 M BICINE, 22.5% PurePEGs PEG 0.3-8 kD | 6.4 |
| 11 | F1 | 0.3 M Calcium chloride, 0.1 M BIS-TRIS propane, 22.5% PurePEGs PEG 0.3-8 kD | 8.5 |
| 12 | F2 | 0.3 M Cobalt(II) chloride hexahydrate, 0.1 M Ammonium citrate, 22.5% PurePEGs PEG 0.3-8 kD | 3.2 |
| 13 | G1 | 0.3 M Di-ammonium hydrogen citrate, 0.1 M Magnesium formate, 22.5% PurePEGs PEG 0.3-8 kD | 4.9 |
| 14 | G2 | 0.3 M DL- malic acid, 0.1 M Sodium cacodylate trihydrate, 22.5% PurePEGs PEG 0.3-8 kD | 2.9 |
| 15 | H1 | 0.3 M Iron(III) chloride hexahydrate, 0.1 M Potassium nitrate, 22.5% PurePEGs PEG 0.3-8 kD | 1.1 |
| 16 | H2 | 0.3 M Lithium chloride, 0.1 M Potassium formate, 22.5% PurePEGs PEG 0.3-8 kD | 6.3 |
| 17 | A6 | 0.3 M Lithium sulfate monohydrate, 0.1 M CHES, 22.5% PurePEGs PEG 0.3-8 kD | 9.5 |
| 18 | A7 | 0.3 M Magnesium acetate tetrahydrate, 0.1 M Citric acid, 22.5% PurePEGs PEG 0.3-8 kD | 4.7 |
| 19 | B6 | 0.3 M Magnesium chloride hexahydrate, 0.1 M Sodium cacodylate trihydrate, 22.5% PurePEGs PEG 0.3-8 kD | 5.8 |
| 20 | B7 | 0.3 M Magnesium nitrate hexahydrate, 0.1 M HEPES, 22.5% PurePEGs PEG 0.3-8 kD | 7.4 |
| 21 | C6 | 0.3 M Magnesium sulfate heptahydrate, 0.1 M Potassium formate, 22.5% PurePEGs PEG 0.3-8 kD | 6.1 |
| 22 | C7 | 0.3 M Nickel(II) chloride hexahydrate, 0.1 M MES monohydrate, 22.5% PurePEGs PEG 0.3-8 kD | 5.7 |
| 23 | D6 | 0.3 M Potassium bromide, 0.1 M BIS-TRIS, 22.5% PurePEGs PEG 0.3-8 kD | 6.6 |
| 24 | D7 | 0.3 M Potassium phosphate monobasic, 0.1 M Tris, 22.5% PurePEGs PEG 0.3-8 kD | 6.1 |
| 25 | E6 | 0.3 M Potassium sodium tartrate tetrahydrate, 0.1 M BICINE, 22.5% PurePEGs PEG 0.3-8 kD | 8.8 |
| 26 | E7 | 0.3 M Potassium tartrate, 0.1 M Imidazole, 22.5% PurePEGs PEG 0.3-8 kD | 7.3 |
| 27 | F6 | 0.3 M Potassium thiocyanate, 0.1 M Ammonium citrate, 22.5% PurePEGs PEG 0.3-8 kD | 5.8 |
| 28 | F7 | 0.3 M Sodium bromide, 0.1 M Sodium cacodylate trihydrate, 22.5% PurePEGs PEG 0.3-8 kD | 6.5 |
| 29 | G6 | 0.3 M Sodium chloride, 0.1 M Tris, 22.5% PurePEGs PEG 0.3-8 kD | 8.1 |
| 30 | G7 | 0.3 M Sodium fluoride, 0.1 M CHES, 22.5% PurePEGs PEG 0.3-8 kD | 9.6 |
| 31 | H6 | 0.3 M Sodium formate, 0.1 M Citric acid, 22.5% PurePEGs PEG 0.3-8 kD | 4.5 |
| 32 | H7 | 0.3 M Sodium iodide, 0.1 M Sodium acetate trihydrate, 22.5% PurePEGs PEG 0.3-8 kD | 4.7 |
| 33 | A11 | 0.3 M Sodium malonate, 0.1 M Sodium citrate tribasic dihydrate, 22.5% PurePEGs PEG 0.3-8 kD | 6.8 |
| 34 | A12 | 0.3 M Sodium nitrate, 0.1 M MES monohydrate, 22.5% PurePEGs PEG 0.3-8 kD | 6.1 |
| 35 | B11 | 0.3 M Sodium phosphate monobasic monohydrate, 0.1 M BIS-TRIS, 22.5% PurePEGs PEG 0.3-8 kD | 5.8 |
| 36 | B12 | 0.3 M Sodium sulfate, 0.1 M BIS-TRIS propane, 22.5% PurePEGs PEG 0.3-8 kD | 9.3 |
| 37 | C11 | 0.3 M Sodium thiocyanate, 0.1 M HEPES, 22.5% PurePEGs PEG 0.3-8 kD | 7.8 |
| 38 | C12 | 0.3 M Succinic acid, 0.1 M Imidazole, 22.5% PurePEGs PEG 0.3-8 kD | 3.4 |
| 39 | D11 | 0.3 M Tri-methylamine N-oxide, 0.1 M BICINE, 22.5% PurePEGs PEG 0.3-8 kD | 8.6 |
| 40 | D12 | 0.3 M Tri-potassium citrate, 0.1 M BIS-TRIS propane, 22.5% PurePEGs PEG 0.3-8 kD | 9.8 |
| 41 | E11 | 10 % Jeffamine M-600, 0.1 M HEPES, 22.5% PurePEGs PEG 0.3-8 kD | 7.5 |
| 42 | E12 | 0.3 M Zinc chloride, 0.1 M Magnesium formate, 22.5% PurePEGs PEG 0.3-8 kD | 5.7 |
| 43 | F11 | 0.3 M Zinc sulfate heptahydrate, 0.1 M Sodium citrate tribasic dihydrate, 22.5% PurePEGs PEG 0.3-8 kD | 4 |
| 44 | F12 | 0.1 M CHES, 22.5% PurePEGs PEG 0.3-8 kD | 9.9 |
| 45 | G11 | 0.1 M Imidazole, 22.5% PurePEGs PEG 0.3-8 kD | 6.6 |
| 46 | G12 | 0.1 M Magnesium formate, 22.5% PurePEGs PEG 0.3-8 kD | 5.9 |
| 47 | H11 | 0.1 M Sodium acetate trihydrate, 22.5% PurePEGs PEG 0.3-8 kD | 4.7 |
| 48 | H12 | 0.1 M Tris, 22.5% PurePEGs PEG 0.3-8 kD | 8.1 |

The SuperCOMBI screen is a reformatting of the SuperSMART and PurePEGs screens onto a single 96-well plate to facilitate regular screening in a 96-experiment format. The position of each crystallization condition is illustrated in the well-map below.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | MSS-1 | MSS-2 | MSS-3 | MSS-4 | MSS-5 | MSS-6 | MSS-7 | MSS-8 | MSS-9 | MSS-10 | MSS-11 | MSS-12 |
| B | MSS-13 | MSS-14 | MSS-15 | MSS-16 | MSS-17 | MSS-18 | MSS-19 | MSS-20 | MSS-21 | MSS-22 | MSS-23 | MSS-24 |
| C | MSS-25 | MSS-26 | MSS-27 | MSS-28 | MSS-29 | MSS-30 | MSS-31 | MSS-32 | MSS-33 | MSS-34 | MSS-35 | MSS-36 |
| D | MSS-37 | MSS-38 | MSS-39 | MSS-40 | MSS-41 | MSS-42 | MSS-43 | MSS-44 | MSS-45 | MSS-46 | MSS-47 | MSS-48 |
| E | MLY-1 | MLY-2 | MLY-3 | MLY-4 | MLY-5 | MLY-6 | MLY-7 | MLY-8 | MLY-9 | MLY-10 | MLY-11 | MLY-12 |
| F | MLY-13 | MLY-14 | MLY-15 | MLY-16 | MLY-17 | MLY-18 | MLY-19 | MLY-20 | MLY-21 | MLY-22 | MLY-23 | MLY-24 |
| G | MLY-25 | MLY-26 | MLY-27 | MLY-28 | MLY-29 | MLY-30 | MLY-31 | MLY-32 | MLY-33 | MLY-34 | MLY-35 | MLY-36 |
| H | MLY-37 | MLY-38 | MLY-39 | MLY-40 | MLY-41 | MLY-42 | MLY-43 | MLY-44 | MLY-45 | MLY-46 | MLY-47 | MLY-48 |

The SuperSMART screen is available in two storage formats. MSS-1 uses a Kisker full-skirted PCR plate with 8-cap strips providing an easy method for long term storage and repetitive usage. This format is NOT compatible with the Mosquito robot from TTP LabTech. MSS-1M uses a U-bottom Greinger assay plate with hear seal specifically selected for Mosquito compatibility. Please contact support@microlytic.com to confirm the format which is best matched to you robotic dispensing needs

| SuperCOMBI Screen - Ordering Information | | | |
|---|---|---|---|
| Cat. No | Product Name | Description | List Price (USD) |
| SSC-1 | SuperCOMBI | SuperSMART and PurePEGs | $195 |
| SSC-1-M | SuperCOMBI for Mosquito | SuperSMART and PurePEGs | $195 |


# Microlytic

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