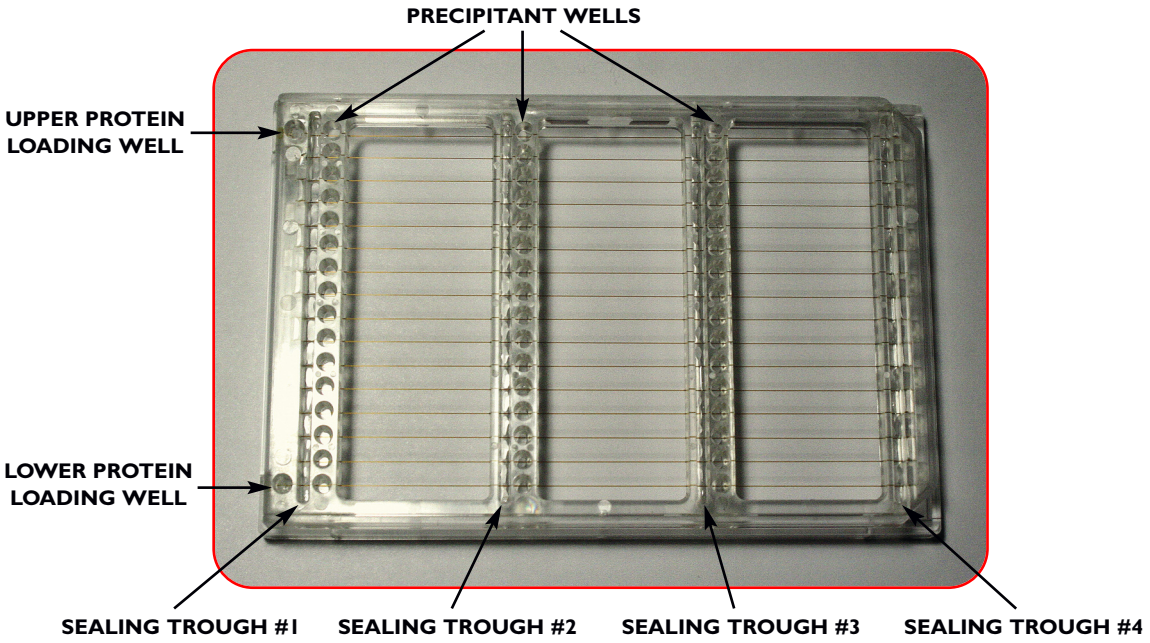
Crystal Harp™ Plate User Guide

SWISSCI

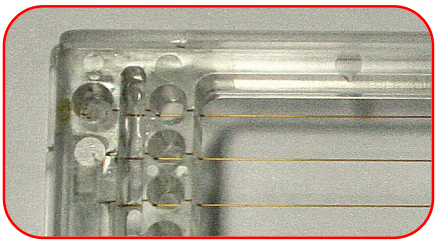
INTRODUCTION

The **CrystalHarp™** plate is designed for crystallization based on capillary diffusion and can be used for crystallization screening and optimization.
Capillary diffusion achieves a much broader screening of variables in one single experiment. The **CrystalHarp™** plate contains 48 capillaries in total and in an ANSI/SLAS 1-2004-Standard format to facilitate handling and imaging.
Addition of cryoprotectants or derivatives for phasing studies can be easily added after crystal appearance. The SBS format enables the usage of the plate directly on beam line robots or alternatively single capillaries can be easily mounted to standard magnetic base, enabling in-situ diffraction analysis.
The unique capillary material allows data collection at room temperature. Flash-freezing in a liquid nitrogen stream (with or without the use of cryoprotectants) is also feasible. The formation of ice-rings is kept to a manageable minimum.



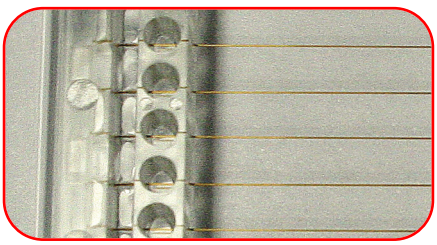
FEATURES

- Capillary diffusion offers a broad variable screen in one experiment
- 48 experiments per plate
- Direct beam line and in situ diffraction analysis possible
- The plate may be vertically mounted
- Unique capillary material with easy to remove crystal methodology



CONTENTS OF BOX

1. **CrystalHarp™** plate
2. Sealant in 10ml syringe with orange delivery nozzle
3. 2ml syringe and flat green nozzle for capillary cleaving
4. Instruction booklet



PRODUCT CODE

CrystalHarp™ Plate - Code QH48T-G100

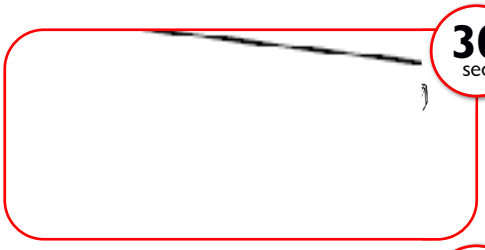
sales@swissci.com

Crystal Harp™ Plate Quick Set Up User Guide

In less than 10 minutes

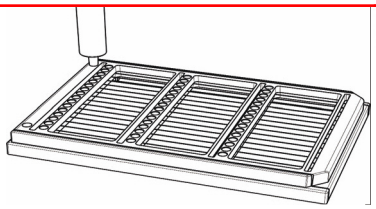
SWISSCI

1 — 30 sec



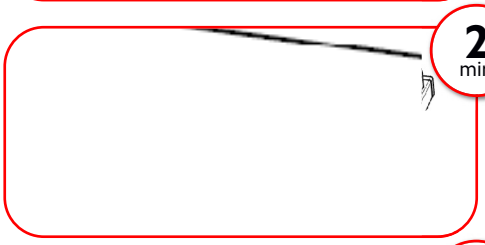
Pipette 22-23 µl of protein solution (blue) into the lower protein loading bay.

2 — 2 min



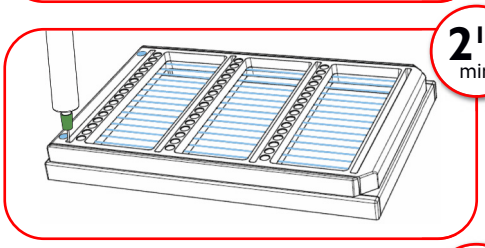
Apply a slight vacuum onto the upper empty protein loading bay with the 2ml syringe. The protein runs quickly through the capillary system.

3 — 2 min



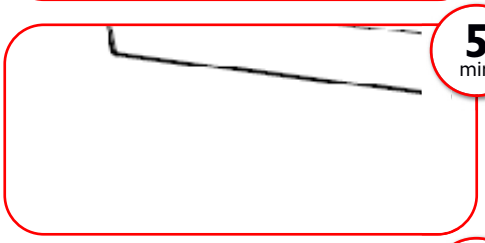
Monitor the filling of the capillary with a microscope. Remove excess protein sample from the protein loading bay.

4 — 2 1/2 min



Break all capillaries in the first left hand sealing trough #1 using the 2ml syringe with flat green nozzle.

5 — 5 min

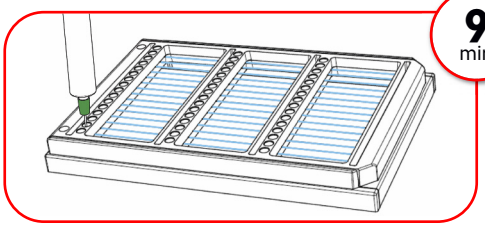


Put the sealant (shown as red) using the 10ml syringe needle into the first long trough.
Repeat step 4 and 5 for the three remaining troughs #2,#3 and #4, one at a time.

6 — 8 min

Pipette 20 µl per well of crystallization condition (shown as grey) into the precipitation wells.

7 — 9 min



Break the capillary in the 48 precipitation wells using the flat ended green syringe one by one. The breaking is audible and can be checked with a microscope.
The precipitation wells need to be sealed to avoid evaporation with a drop of mineral oil to each well or using the Swissci sealant.
The **CrystalHarp** plate is ready for incubation.

Crystal Harp™ Plate - Code QH48T-G100

sales@swissci.com

USER INSTRUCTIONS

1. Remove the plate carefully from the box. Remove the protective cover and place the plate in the orientation as per **Figure 1** with the cut corners to the right hand side.

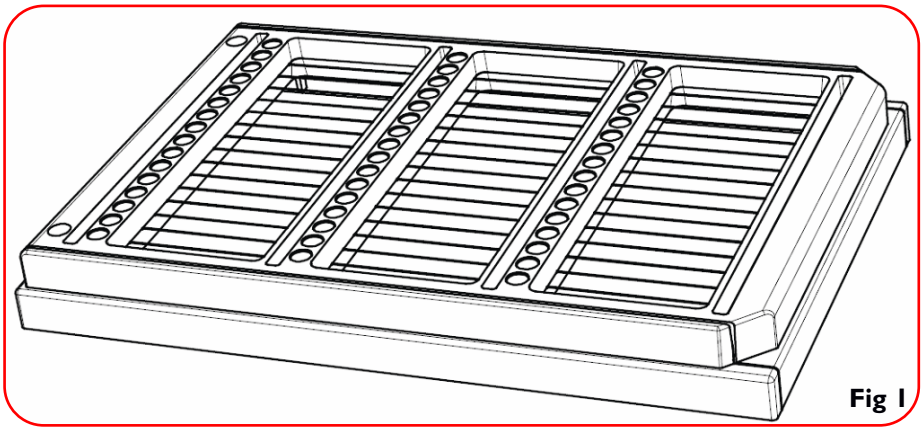


Figure 1

2. Pipette 22-23 µl of protein solution into the lower protein loading bay located on the bottom left hand side of the plate (**Figure 2**).

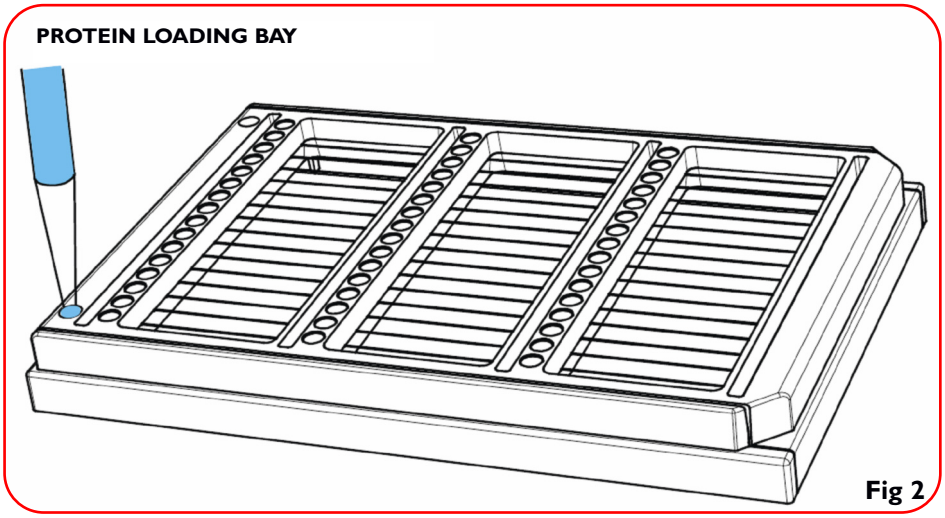


Figure 2

sales@swissci.com

3. Using the 2ml syringe apply a slight vacuum on the upper loading well NOT containing the protein solution to ensure the protein runs quickly through the capillary system (**Figure 3**). Too high of a vacuum may cause air bubbles to form in the protein solution.

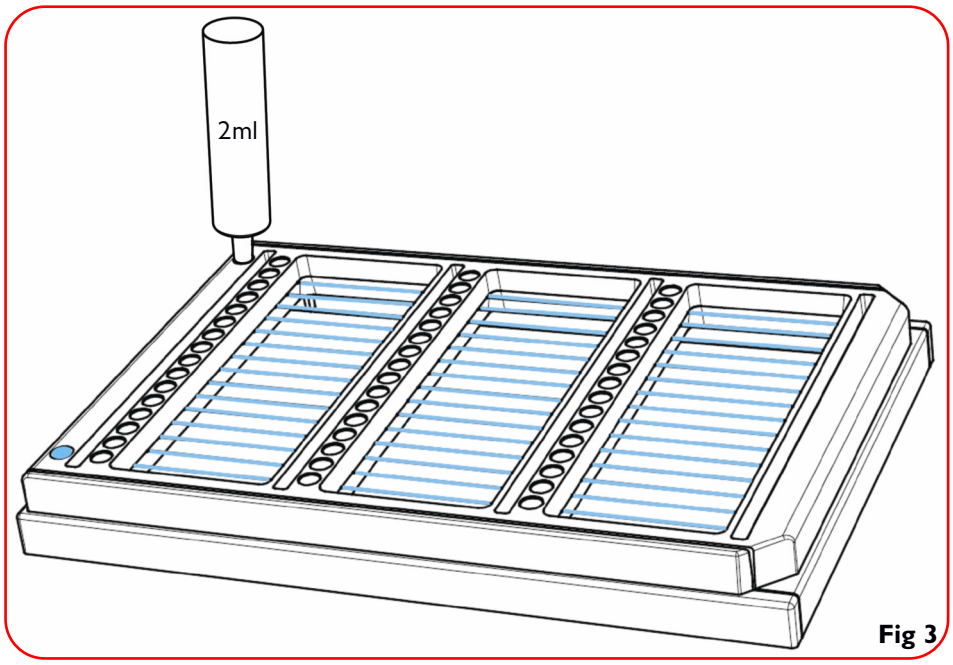


Figure 3

4. The filling of the capillary can be easily monitored with a microscope. Release the vacuum when the capillary is fully loaded (**Figure 4**). Excess protein can be removed from the lower protein loading well.

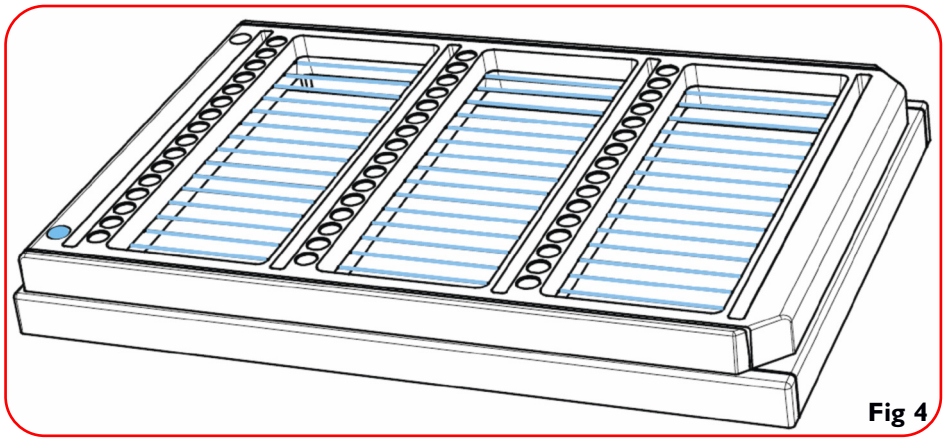


Figure 4

sales@swissci.com

5. In case the protein has run too far through the capillary, indicated by an air bubble in the capillary near the protein loading bay, simply apply vacuum on the original protein loading well. (**Figure 5**).

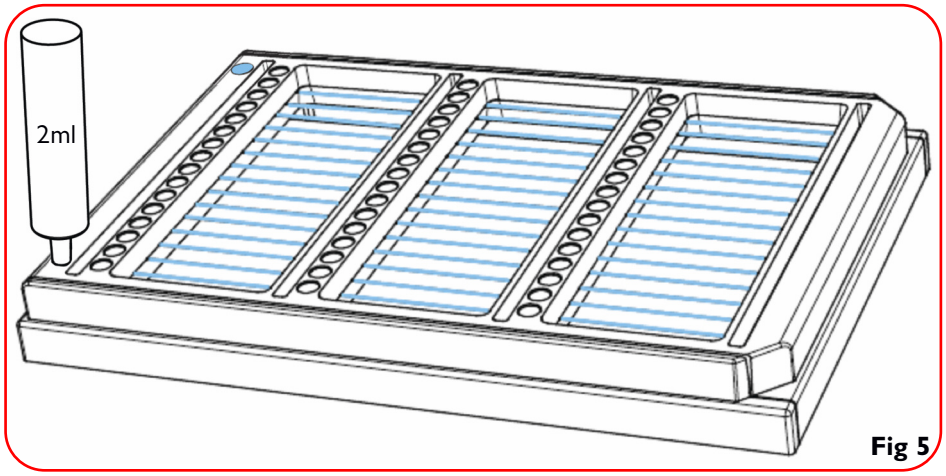


Figure 5

6. Break each of the 16 capillaries in the first long trough #1 with the 2ml syringe and flat green nozzle for capillary cleaving. (**Figure 6**).

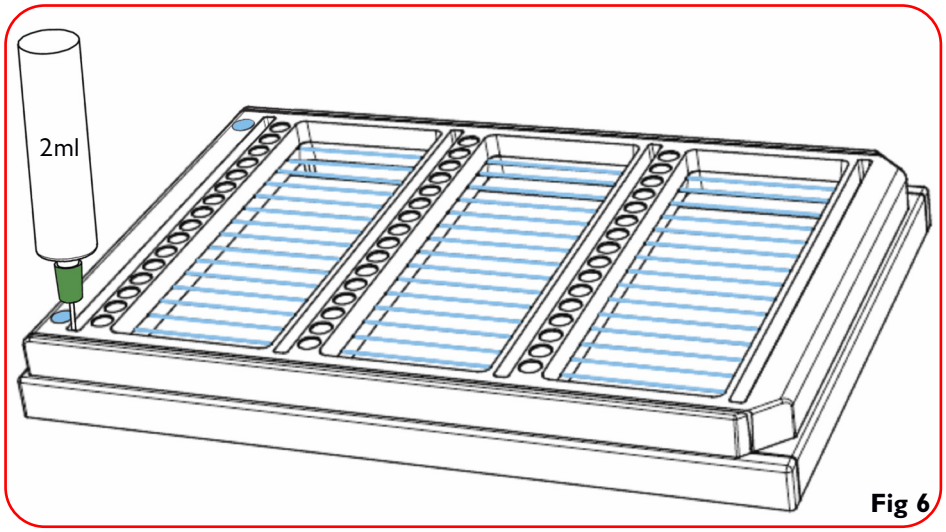


Figure 6

7. Insert the sealant sealing paste using the syringe (10ml size) with the orange delivery nozzle into the first long trough #1 (**Figure 7**). Ensure the sealing paste completely encloses each broken end of the capillaries without any entrapped air bubble. Fill the entire trough to the top with sealing paste.

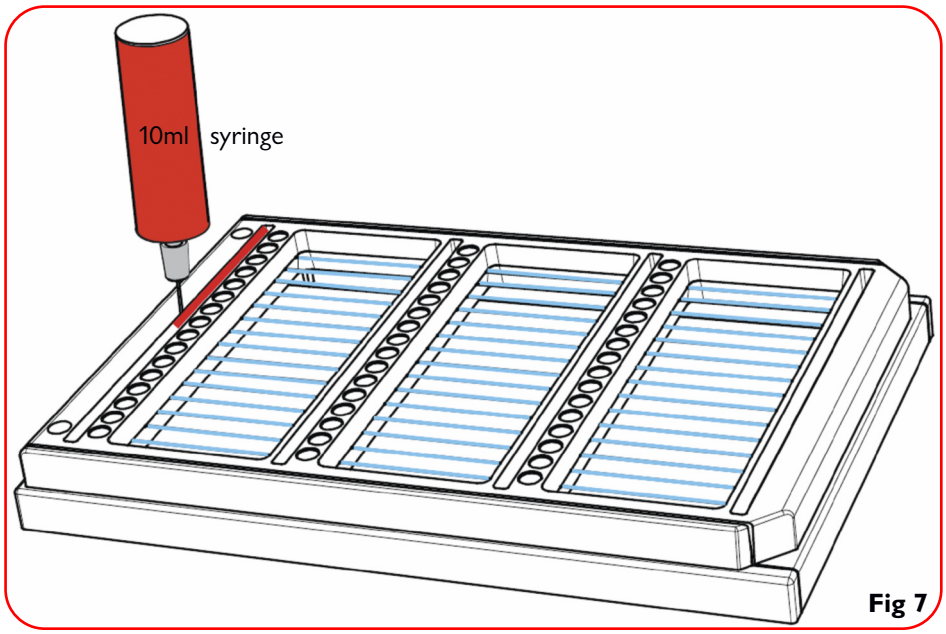


Figure 7

Then repeat step 6 and 7 for the three remaining troughs (#2,#3 and #4) one at a time with the flat nozzle and sealing paste.

8. Pipette 20 µl per well of crystallization condition into the precipitation wells as shown in **Figure 8**. To expedite this step a multi-channel 8-way pipette can be used. Please note that the first tip enters the well #1 and the second will be in #3 etc. And a second pipetting operation is used to fill wells 2, 4, 6 etc. Use the **CrystalHarp™** plate scoring sheet to register the location of each condition. Ensure no air bubble is trapped on the bottom of the well.

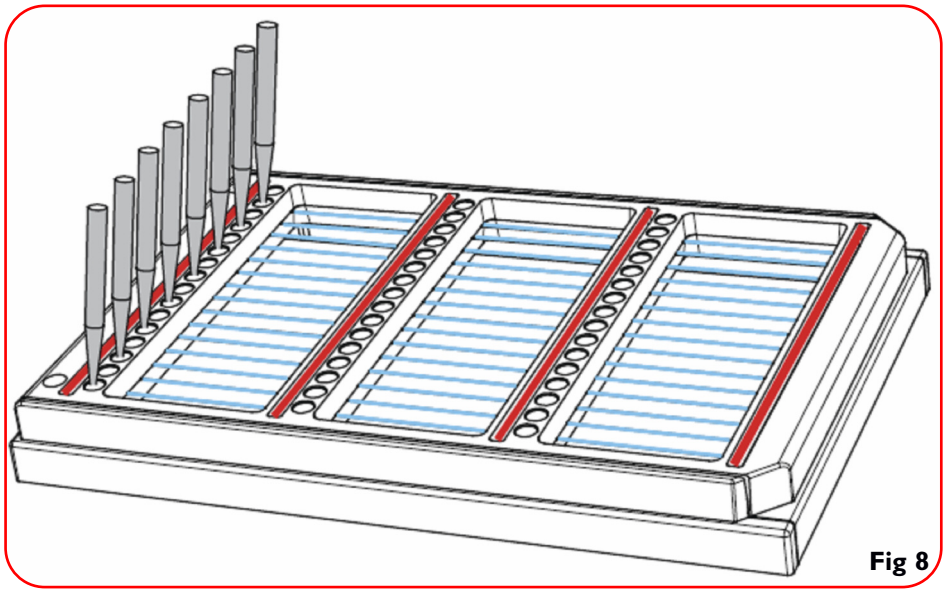


Figure 8

sales@swissci.com

9. After filling all 48 precipitation wells use the Capillary Cleaving flat end green nozzle of 2ml syringe to cut the capillary of those 48 wells, one capillary at a time. Hold the flat green nozzle into the filled precipitation well and gently twist. The breaking of capillary is audible and can be also checked with a microscope (**Figure 9**). Avoid cross contamination by cleaning after each cut with tissue wipe.

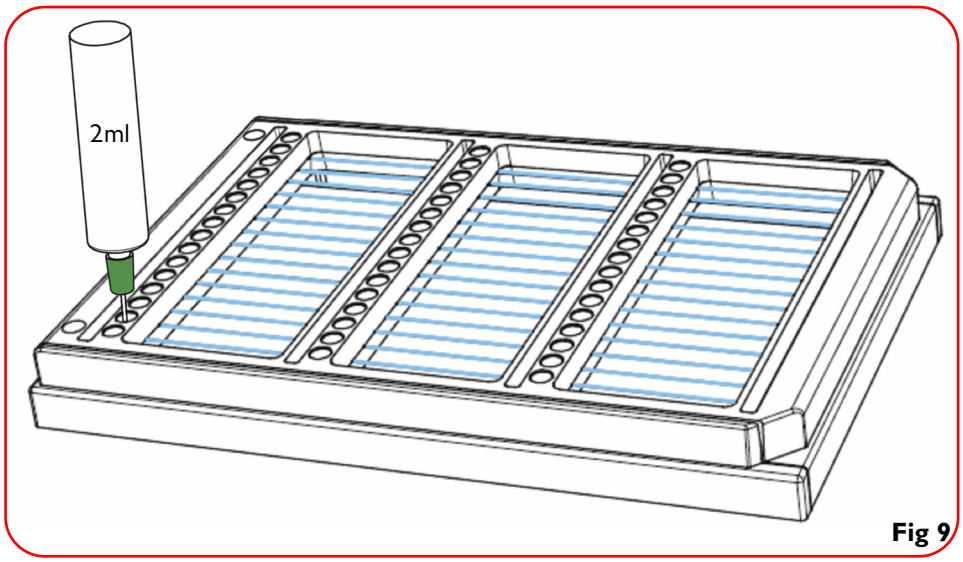


Figure 9

10. The 48 precipitation wells need to be sealed to avoid evaporation. This can simply be done by adding a drop of mineral oil to each well or by adding a drop of the SWISSCI sealant.

11. The **CrystalHarp™** plate is now ready for incubation at the desired temperature between 4° and 25°C.

CrystalHarp™ Plate Scoring Sheet

Plate#		Date	
Protein ID		Screen ID	
		Temperature	

Capillary#	Well Condition										
1											1
2											2
3											3
4											4
5											5
6											6
7											7
8											8
9											9
10											10
11											11
12											12
13											13
14											14
15											15
16											16
17											17
18											18
19											19
20											20
21											21
22											22
23											23
24											24
25											25
26											26
27											27
28											28
29											29
30											30
31											31
32											32
33											33
34											34
35											35
36											36
37											37
38											38
39											39
40											40
41											41
42											42
43											43
44											44
45											45
46											46
47											47
48											48

ADDITIONAL INFORMATION

IMAGING and X-RAY ANALYSIS

Imaging

Manual inspection of crystal growth can be performed using a microscope. Alternatively the SBS formatted **CrystalHarp™** plate allows for commercial automatic incubation and the use of imaging systems.

X-ray Diffraction Analysis

For in-situ X-ray diffraction analysis the **CrystalHarp™** plate can be used with an X-ray Scanner or with a plate handling robot at a beam line. If the entire plate is to be mounted, the oil filled wells need to be completely full. After this a UV slide will be placed across to ensure no bubble are present. Following this the combined slide and plate are sealed together with tape and ready for in-situ analysis. Please contact sales@swissci.com for more information.

Alternatively diffraction studies can be performed on single capillaries. For that the desired capillary segment is removed from the plate using a razor blade or a pair of small scissors. It is recommended to first cut the end near the precipitation well to avoid uncontrolled movement of the liquid in the capillary. The cut capillary segment is then placed into a metal tube (ID 0.2mm) that has been pre-mounted to a standard magnetic base (**Figure A**).

Magnetic base mounts (Sample Holder Caps) are available from either Hampton (HR4-733) or Molecular Dimensions (MD7-400).

CrystalHarp™ plate is available from Molecular Dimensions (MD11-57).

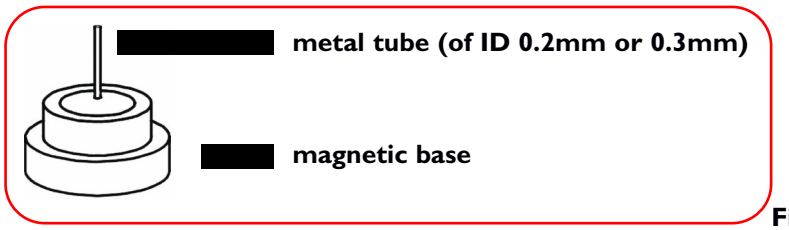


Fig A

Figure A

Mounting and X-ray studies under ambient conditions

If the capillary is not flash-frozen in liquid nitrogen (i.e. for transport or storage) we recommend sealing one end of the capillary segment with wax. The unsealed end is placed into the pre-mounted metal tube and a drop of e.g. super glue applied to fix the capillary segment containing the crystals (**Figure B**).

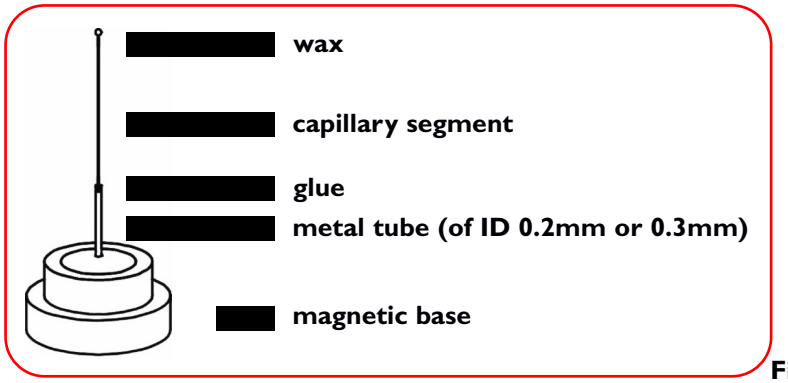


Fig B

Figure B

Mounting and X-ray studies under flash-frozen conditions
If the crystals in the cut out capillary segment need to be flash-frozen the standard magnetic base and metal tube are used without wax sealing (**Figure C**).

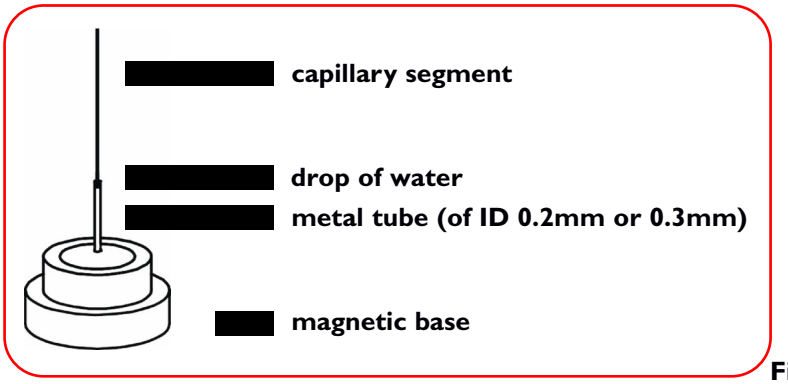


Fig C

Figure C

For mounting the capillary segment, the pre-mounted metal tube has to contain a small amount of water. The cut capillary segment is then stuck into the metal capillary without the addition of any glue or sealing the top end. The mounted capillary can now be flash-frozen in liquid nitrogen in the customary way, i.e. for freezing a loop. For placing the mounted capillary onto the goniometer it is recommended to use a cryo tong designed for large pin heights (24 mm).

sales@swissci.com