

Easy set-up for high throughput protein crystallization experiments using the VIAFLO 96/384 handheld electronic pipette

Introduction

Protein crystallization and subsequent X-ray analysis have contributed to major advances in the determination of protein structures. Successfully growing protein crystals is challenging and depends on many factors, such as the nature of the reagents, the pH and the temperature. The sitting drop vapor diffusion technique is a protein crystallography method that is very well suited to a 96 well crystallization plate format.

All wells of a plate of this type can be filled simultaneously with perfect tip alignment using the VIAFLO 96/384 handheld electronic pipette with a 96 channel pipetting head. Using a single aspiration step reduces the time to cover the entire well plate, which helps to prevent evaporation of precious samples, resulting in more homogeneous crystal growth across the plate.

Key benefits:

- Accommodate and process 96 well crystallization plates on the VIAFLO 96/384 for high throughput crystallization screens.
- Increased pipetting precision when pipetting into reservoir or crystallization wells.
- The VIAFLO 96/384 handheld electronic pipette allows easy and rapid set-up of up to 96 different crystallization conditions for increased productivity and reproducibility.
- Optimal pipetting heights for transfer of proteins and reagents directly into the crystallization plate are guaranteed.
- The VIAFLO 96/384 allows for fast set-up of crystallization reactions, which helps to avoid evaporation effects, resulting in more robust crystal growth.

Step-by-step procedure:

Experimental set-up

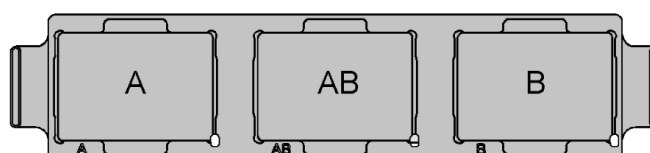
The following protocol describes a set-up for performing an initial protein crystallization screen using the VIAFLO 96/384 handheld electronic pipette together with a three position stage (**Figure 1**).

In this protocol, a 96 deep well block filled with 96 different reagents (Morpheus® HT-96, Molecular Dimensions), placed on **Position A**, is used as the source for a 96 condition crystallography screen. The protein crystallography plate (3 Lens Crystallisation Microplate (UVXPO), SWISSCI) is placed on **Position AB**, while tips and the tip waste are accommodated on **Position B**. Three different protein solutions are filled into PCR strips and accommodated on a PCR cooling block, which is exchanged with the Morpheus HT-96 screen plate as required.

VIAFLO 96/384



Three position stage



A 96 channel pipetting head (2-50 µl) is used together with 125 µl Sterile, Filter, Low Retention GripTips. Customized VIALINK programs are provided to set up a protein crystallization screen with the VIAFLO 96/384 handheld electronic pipette.

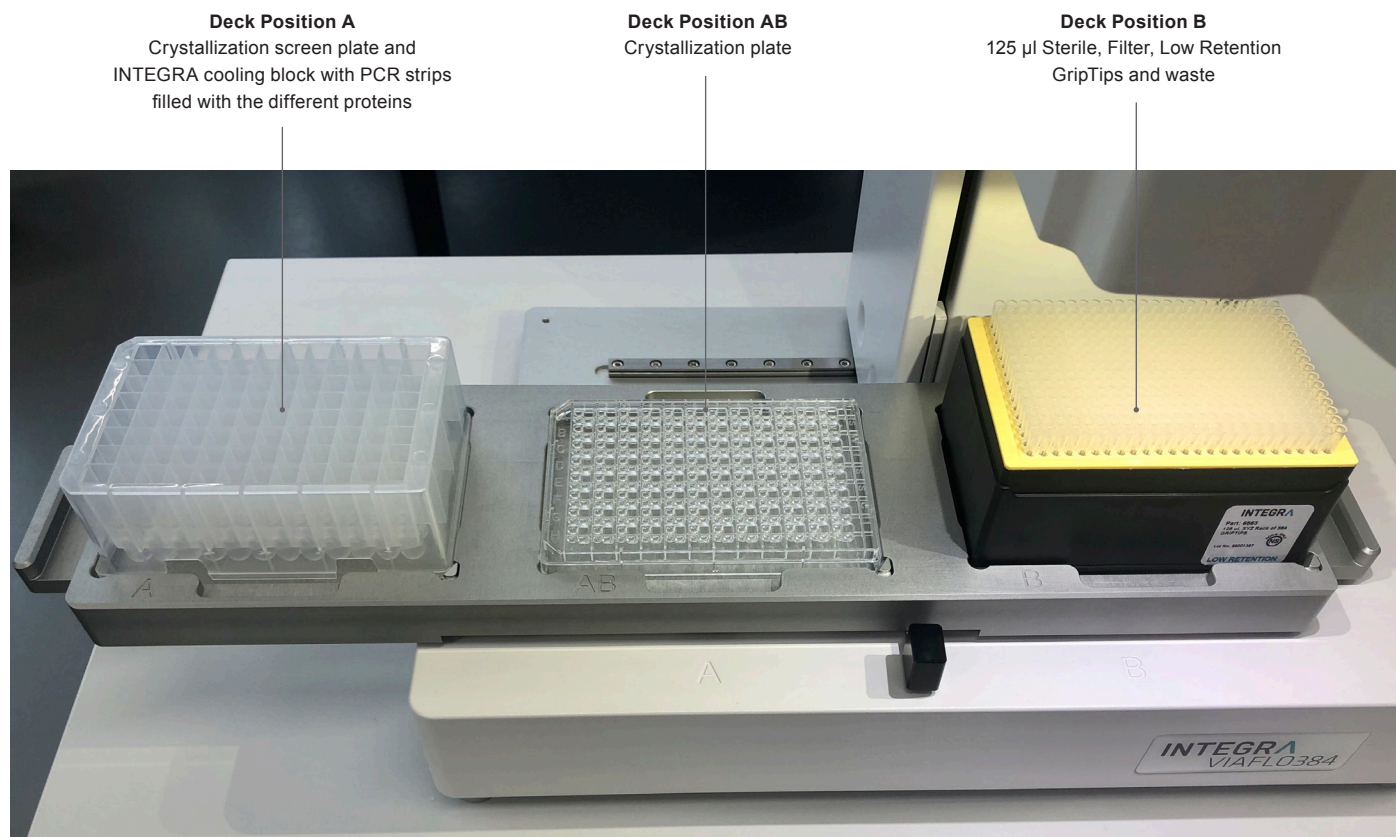


Figure 1: The VIAFLO 96/384 handheld electronic pipette with a three position stage showing the experimental set-up. **Position A:** crystallization screen plate. **Position AB:** crystallization plate. **Position B:** 125 µl Sterile, Filter, Low Retention GripTips and waste to collect the discarded tips.

1. Transfer of the screening agents

STEP: Transfer the crystallization screening agents into each well of the crystallization plate

HOW TO: Load tips and launch the program 'CRYST_SCREEN' to fill the lens and reservoir wells (Figure 2) of the crystallization plate with the crystallization screening agents.

Aspirate 40 µl of the Morpheus HT-96 reagent and dispense 2 µl back into the 96 deep well block (**Position A**), as indicated in the pipetting program. Dispense 2 µl into each lens well (1-3), and 30 µl into the reservoir well (4), of the crystallization plate on **Position AB** according to **Figure 2**. Move the plate slider of the three position stage to access the different wells of the crystallization plate more easily. Discard the pipette tips with the remaining liquid. The accuracy of these pipetting steps is increased by using a pre- and post-dispense.

Tips:

- Pipetting heights are defined in the program to prevent the tips from touching the bottom of the plate.
- The volumes dispensed can be easily adjusted to allow investigation of different ratios of protein and precipitant.

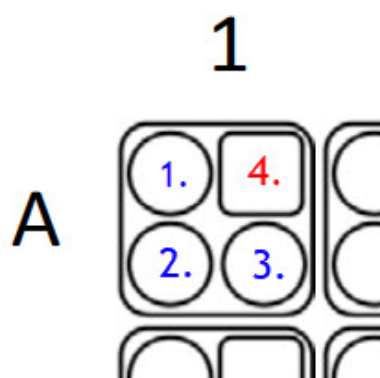


Figure 2: Pipetting scheme of the crystallization plate well. Lens wells: 1-3. Reservoir well: 4.

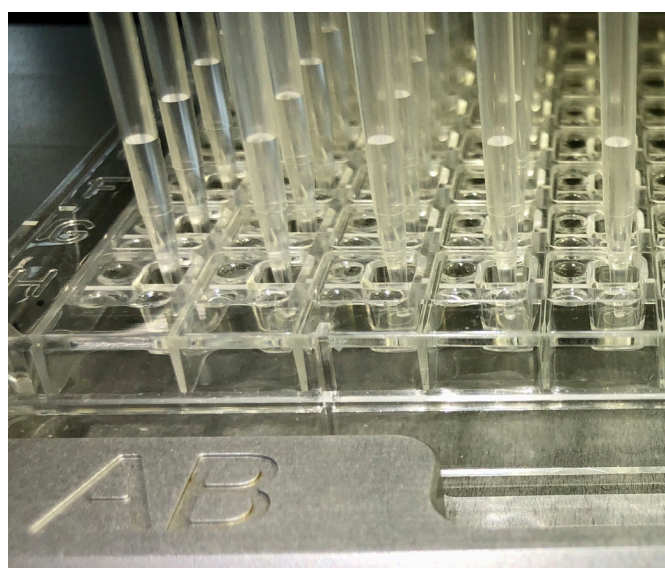
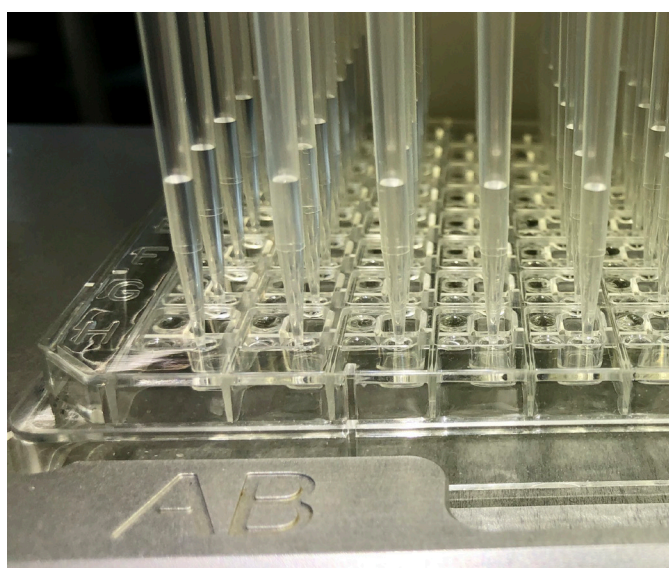
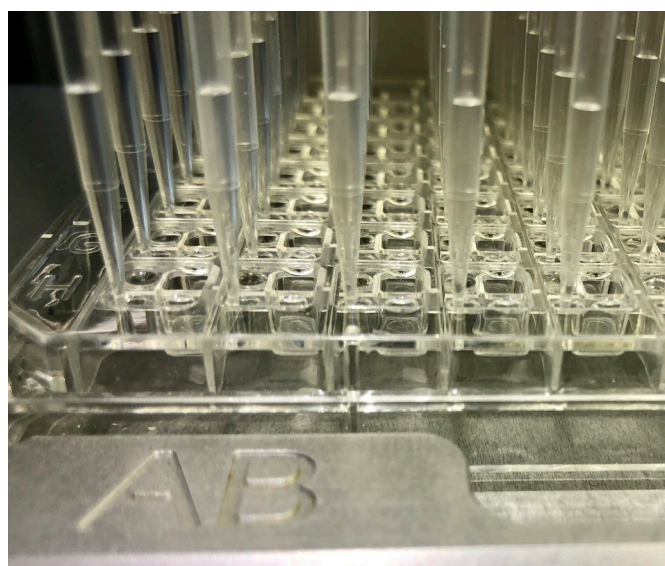
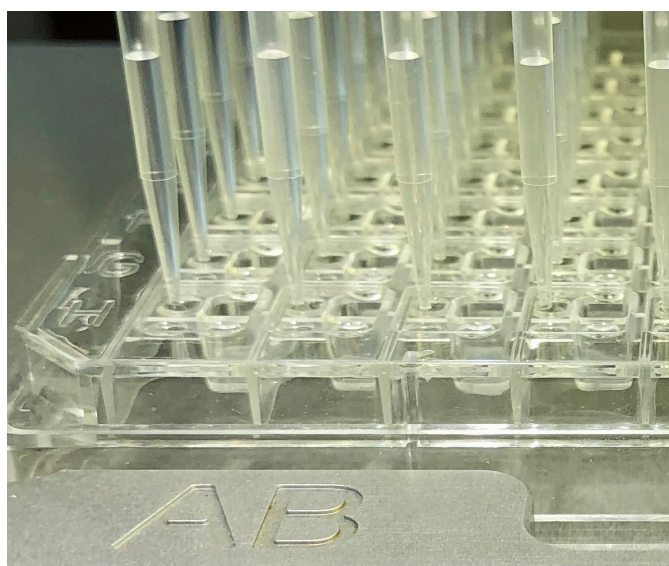


Figure 3: All four wells (three lens wells and one reservoir well) of the crystallization plate are accessible with the VIAFLO 96/384 handheld electronic pipette.

2. Transfer of protein solutions

STEP: Transfer up to three different protein solutions into the wells of the crystallization plate.

HOW TO: 30 μ l of each of the three different protein solutions are manually distributed into 8 tubes of a PCR strip to allow subsequent aspiration with 8 pipette channels in parallel. The PCR strip containing protein solution 1 is positioned in column 12 of a cooling block, which acts as a support. Instead of loading all 96 tips at once, load 8 tips to the last column of the pipetting head and aspirate 28 μ l of protein solution 1. Perform a pre-dispense (2 μ l) and then transfer 2 μ l to lens well 1 (**Figure 2**) of each column of the crystallization plate (**Figure 4**). Perform a post-dispense (2 μ l). Discard the tips and exchange the PCR strip for one containing protein solution 2. Transfer the protein solution into lens well 2. Repeat the process for protein solution 3 and lens well 3.

Tips:

- Aspiration and dispense speeds can be easily adapted to allow optimized handling of the protein solutions.
- The column detect mode of the VIAFLO 96/384 handheld electronic pipette offers users haptic feedback for accurate and easier guidance when moving to the next column.
- Protein solutions can be kept chilled in the cooling block if necessary.
- Protein solutions can also be stored in a 12 column reagent reservoir if higher volumes of the protein solution are available. The corresponding VIALINK program can be found in the download section.
- Alternatively, 2 μ l of protein solution can be transferred from a PCR strip into the crystallization wells using a VOYAGER 8 channel 12.5 μ l adjustable tip spacing electronic pipette with 12.5 μ l Sterile, Filter, Low Retention GripTips.

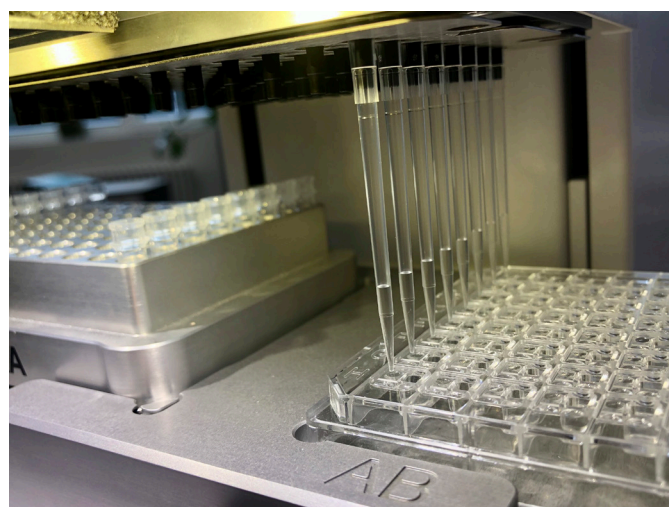
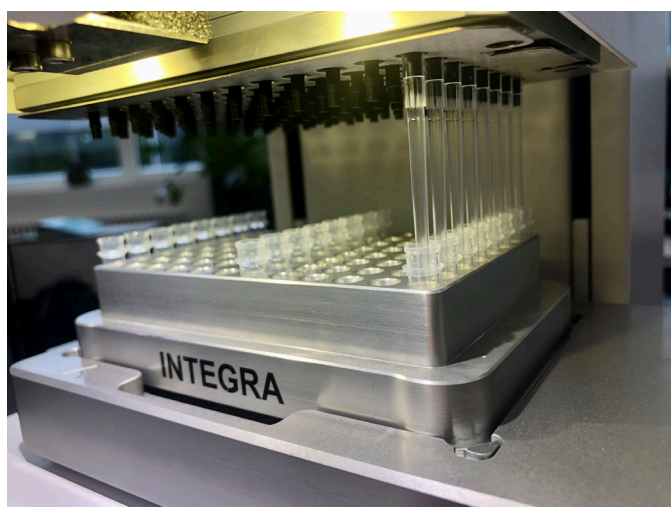


Figure 4: Pipetting and transfer of the protein solution with the VIAFLO 96/384 handheld electronic pipette.

3. Sealing of the crystallization plate**STEP:** Seal and incubate the crystallization plate.**Tip:**

- The duration and temperature of sitting drop vapor diffusion depends on your proteins.

Conclusion

- The VIAFLO 96/384 handheld electronic pipette allows transfer of liquids to crystallization plates in a fast and easy way for high throughput screening of 96 crystallization conditions.
- Optimal consistency and reproducibility of the results are achieved by using the VIAFLO 96/384 to simultaneously transfer 96 liquids.
- The protocol for different protein solutions and crystallization screens can be easily adapted using the VIAFLO 96/384.
- Crystallization plates for sitting drop vapor diffusion in the ANSI/SLAS plate format are optimal for processing with the VIAFLO 96/384 handheld electronic pipette.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	6001/6031	VIAFLO 96 or 384 handheld electronic pipette (base unit)	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96384#downloads
INTEGRA Biosciences	6230	Three position stage for 96 and 384 well plates	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96384#parts-and-numbers
INTEGRA Biosciences	6106	96 channel pipetting head 50 µl	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96384#parts-and-numbers
INTEGRA Biosciences	6362	12 Column Reagent Reservoir	https://www.integra-biosciences.com/switzerland/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
INTEGRA Biosciences	6565	125 µl Sterile, Filter, Low Retention Griptips	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	6250	PCR 96 Well Cooling Block	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
CORNING	CLS 3741	Corning® Thermowell GOLD PCR 8 well tube strips	https://www.sigmaaldrich.com/catalog/product/sigma/cls3741?lang=de&region=CH
SWISSCI	UVXPO-3LEN-SLP	3 Lens Crystallisation Plate	https://swissci.com/wp-content/uploads/2020/03/3-Lens-plate.pdf
MOLECULAR DIMENSIONS	MD1-47	Morpheus HT-96	https://www.moleculardimensions.com/products/morpheus

INTEGRA Biosciences AG
7205 Zizers, Switzerland
T +41 81 286 95 30
F +41 81 286 95 33
info@integra-biosciences.com

INTEGRA Biosciences Corp.
Hudson, NH 03051, USA
T +1 603 578 5800
F +1 603 577 5529
info-us@integra-biosciences.com

INTEGRA Biosciences Deutschland GmbH
35444 Biebertal, Deutschland
T +49 6409 81 999 15
F +49 6409 81 999 68
info-de@integra-biosciences.com

INTEGRA Biosciences SAS
95062 Cergy-Pontoise Cedex 1, France
T +33 (0)1 34 30 76 76
F +33 (0)1 34 30 76 79
info-fr@integra-biosciences.com

INTEGRA Biosciences Ltd.
Egham, Surrey TW20 9EY, UK
info-uk@integra-biosciences.com