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Advanced High-Throughput Platforms for Protein Crystallisation

The most widely used method for the determination of protein structures is the X-ray analysis of protein crystals. This type of analysis has led to major advances in basic research, particularly in the fields of structural genomics, and structure-based drug design.

In recent years the field of protein crystallisation has undergone fundamental changes. There has been a dramatic rise in the demand for sophisticated, specialised and flexible platforms for high-throughput crystallography. These systems need to provide multiple screening capabilities, excellent optical characteristics and be suitable for use with small sample volumes that may or may not contain compounds that lower the surface tension of the sample. Microplates designed to be used with these systems have similarly advanced in both capability and effectiveness. The architecture of these plates facilitates low protein and reagent consumption whilst maintaining compatibility with high-density applications and automated equipment.

Certain unique polymers provide a low birefringent background (LBR) for crystal inspection under polarised light. Microplates can also be treated to produce a hydrophobic surface which prevents the deformation of crystallisation drops in the crystallisation wells. This is especially important when surface tension reducing substances, such as detergents, are used when working with membrane proteins, which enables the use of flat bottom crystallisation plates when investigating membrane proteins.

1. 96 Well CrystalQuick™ Plates for High-Throughput Vapour Diffusion Applications

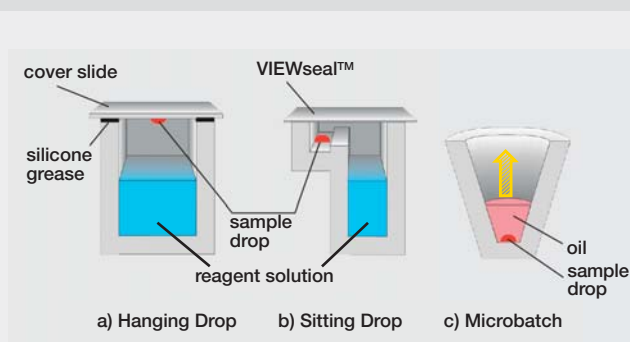


Figure 1: Crystallisation methods
a) Hanging Drop b) Sitting Drop c) Microbatch

The two vapour diffusion techniques, sitting drop and hanging drop, are the most common methods for crystallising proteins. (Fig. 1 a, b). Using these methods, a sample droplet, formed by mixing a protein solution with a reagent solution, is incubated alongside a larger volume of the same reagent solution. Both sample droplet and larger reagent solution are sealed within a single well. The reagent solution can contain a wide range of chemicals, e.g. buffers, salts and precipitation agents. When the sample solution is prepared by mixing this with the protein sample, the concentration of the reagent solution is diluted which initiates a concentration gradient between the sample solution and larger reagent solution within the vapour diffusion well. Equilibrium is then achieved by passive diffusion, and

as this process occurs, the concentration of proteins and chemicals in the crystallisation droplet rises. If the correct conditions have been reached, the process of crystallisation is induced.

Numerous factors affect the crystallisation of proteins and in general cannot be predicted. A large number of experimental conditions often need to be examined before optimal parameters for crystallisation can be defined. Due to the large number of conditions that have to be tested, protein crystallisation still represents a major bottleneck in the determination of protein structures. The use of high-throughput equipment and techniques such as pipetting robots and imaging systems has greatly reduced the time necessary to define the appropriate conditions for crystallisation. The Greiner Bio-One range of CrystalQuick™ microplates for sitting drop applications has been specifically designed to be compatible with a wide range of automated systems.

The CrystalQuick™ microplates (Fig. 2) have been developed in cooperation with the Genomics Institute of the Novartis Research Foundation (GNF) in San

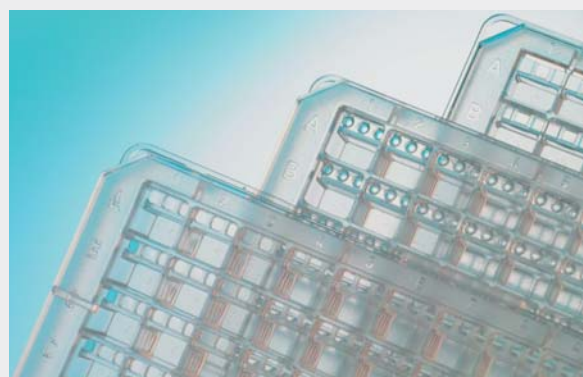


Figure 2: CrystalQuick™ plates with different well profiles

Diego (USA), the Max-Planck Institute (MPI) and the Protein Structure Factory (PSF) in Berlin. The geometry of the plate closely follows that of a standard 96 well microplate. Each reservoir corresponds to a stan-

standard well position but contains an additional elevated platform that consists of one or three sample crystallisation wells. The plates are optimised for sealing with an adhesive film such as VIEWseal™ or AMPLIseal™. All CrystalQuick™ microplates are available in an LBR version (→ chapter 3) for use with polarised light and partially with a hydrophobic surface (→ chapter 4).

CrystalQuick™ SW (Square Wells) (Fig. 3)

With three sample crystallisation wells per reservoir, CrystalQuick™ SW (US Patent No. 7005008 B2) makes it possible to test 288 samples per plate. Each sample crystallisation well allows for a maximum droplet volume of 4 µl and the flat bottom of each well provides good optical clarity.

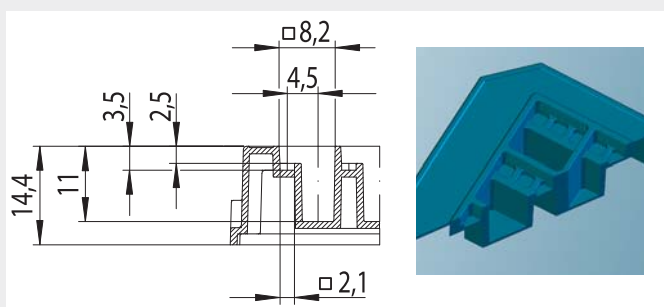


Figure 3 a): Well profile, CrystalQuick™ SW

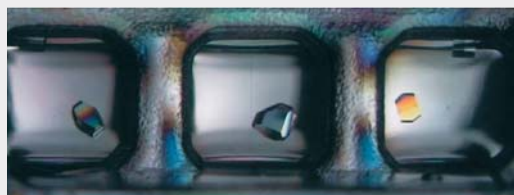


Figure 3 b): Crystallisation of lysozyme in CrystalQuick™ SW

CrystalQuick™ RW (Round Wells) (Fig. 4)

With three round crystallisation wells per reservoir, CrystalQuick™ RW allows 288 samples to be tested per plate. The base of the crystallisation wells are concave and hold a maximum sample droplet volume of 1.9 µl.

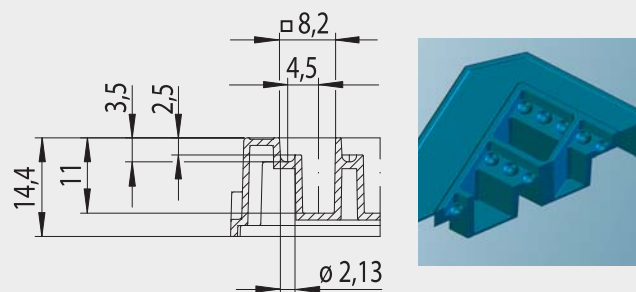


Figure 4 a): Well profile, CrystalQuick™ RW

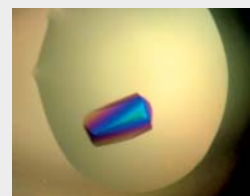


Figure 4 b): Protein crystal in CrystalQuick™ RW, the image was kindly supplied by B. Blattmann, NCCR Structural Biology, Zurich (Switzerland)

CrystalQuick™ LP (Low Profile) (Fig. 5)

Low profile crystallisation plates simplify crystal harvesting due to the angled walls of the crystallisation wells. The low profile of the plates reduces storage requirements and the flat base of the sample crystallisation well provides excellent visual clarity.

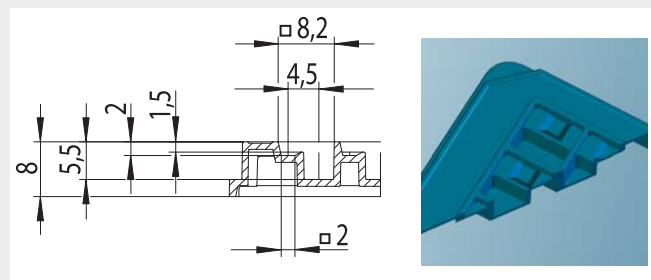


Figure 5 a): Well profile, CrystalQuick™ LP



Figure 5 b): Crystallisation of lysozyme in CrystalQuick™ LP, RoboDesign International Inc., Carlsbad (USA)

CrystalDrop™ Lid (for Hanging Drop Applications)

The combination of CrystalDrop™ lid and Crystal-Quick™ plate makes it possible to conduct up to 288 sitting drop and 192 hanging drop experiments in parallel (Fig. 6). This gives a potential total number of 480 crystallisation drops per plate. The CrystalDrop™ lid allows the placement of the hanging drops over the reservoir well and so eradicating any potential visual overlap from the sitting drop experiments. Channels around each of the 96 crystallisation positions enable the individual sealing of each well with silicone grease.

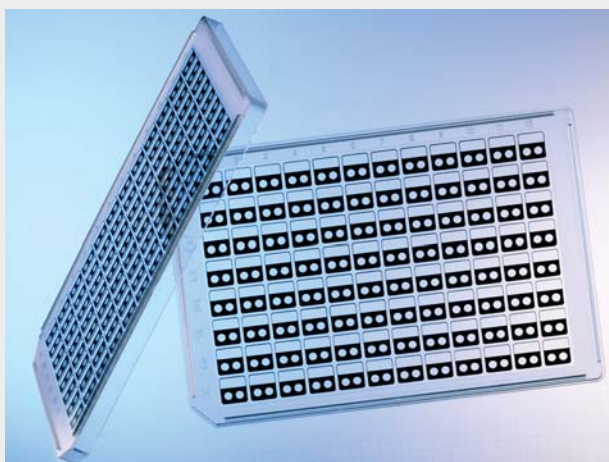


Figure 6: CrystalDrop™ lids

CrystalDrop™ lids are also available pre-greased (Fig. 7) to save time and to provide a perfect sealing.

The CrystalDrop™ lid was developed in cooperation with the Max-Planck Institute and the Protein Structure Factory in Berlin (Germany) specifically for use in high-throughput crystallisation applications.



Figure 7: Pre-greasing of CrystalDrop™ lid

2. IMP@CT™ Plates for Microbatch Applications

In microbatch under oil experiments, crystallisation droplets are located under a layer of oil (Fig. 1c). Paraffin, silicone or a mix of both oils can be used to perform experiments. Paraffin oil is virtually impermeable to water vapour and when used results in a very slow rate of change in droplet conditions. Therefore initial state of the crystallisation droplet remains more or less constant. Silicone oil in contrast, readily allows water vapour diffusion. The use of silicone oil in microbatch techniques results in a continuous increase in concentration of all components contained within the crystallisation droplet. This leads to the ability to examine a wide range of conditions in a single setup. The 96 well IMP@CT™ plates feature a double rimmed perimeter moat for the addition of water, reagent solution or gel to control evaporation in the plate or to perform vapour batch experiments. The microbatch under oil technique is of particular benefit when working with oxygen sensitive proteins as the oil layer protects the protein from potentially damaging atmospheric oxygen.

IMP@CT™ plates are designed for high-throughput crystallisation that utilises microbatch under oil techniques. The wells have a truncated conical geometry with a flat base. This conical shape aids in centrally locating the sample droplet in the well even when small

sample volumes are used. The flat well base facilitates high visual clarity for monitoring the samples.

96 Well IMP@CT™ Plate (Fig. 8, 9)

The 96 well IMP@CT™ plate was developed by Greiner Bio-One in cooperation with Hampton Research (USA) and Allan D'Arcy. The double rim of the plates means that the wells can be filled separately or together with oil. Filling the double rim of the IMP@CT™ plates with an aqueous gel makes it possible

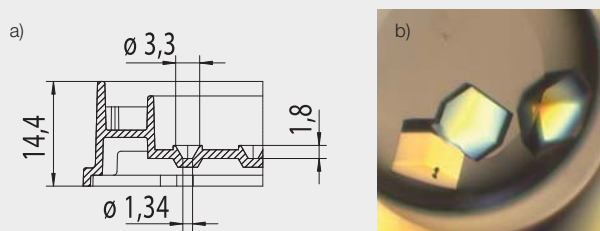


Figure 8 a): Well profile, 96 Well IMP@CT™ plate
b) Protein crystals in the 96 well IMP@CT™ plate

to control water evaporation from the crystallisation drops. The 96 well IMP@CT™ plate is also available as a black μ Clear® version with a film bottom. This plate is characterised by extremely low light scattering between individual wells and by low birefringence.

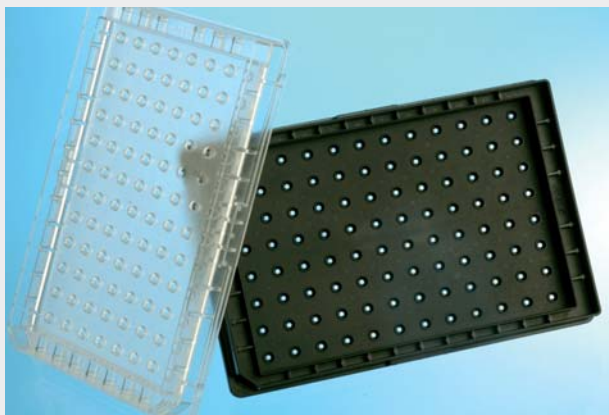


Figure 9: 96 well IMP@CT™ plates

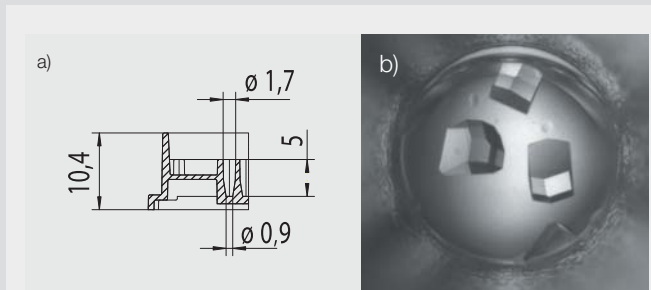
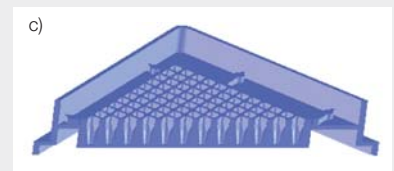


Figure 10 a) + c): Well profile, 1536 well IMP@CT™ plate
b) Crystallisation of lysozyme in the 1536 well IMP@CT™ plate, with the kind approval of the Hauptmann-Woodward Medical Research Institute, Buffalo (USA)



1536 Well IMP@CT™ LBR Plate (Fig. 10, 11)

The 1536 well IMP@CT™ plate was developed in cooperation with the Hauptmann-Woodward Medical Research Institute in Buffalo (USA) for use in automated high-throughput systems. The optimised half-conical well geometry means that even sample droplets with a very small volume are localised at the centre of the wells. Smooth, flat well bottoms provide optimum conditions for visual monitoring and evaluation of the crystallisation samples. The 1536 well IMP@CT™ plate is designed to be used in conjunction with polarised light for improved crystal detection.

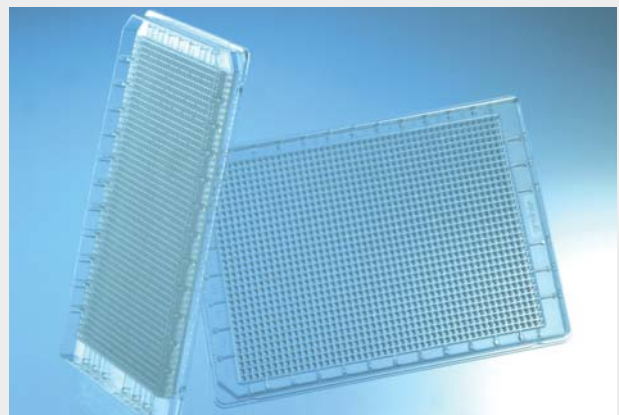


Figure 11: 1536 well IMP@CT™ plates

3. LBR Plates for Polarised Light and UV-Light Detection

An important issue for high-throughput protein crystallisation is the visual inspection of the droplets. Thanks to the birefringent properties of crystalline material, the application of polarised light in crystal identification is a powerful tool. The use of crossed polarisers in imaging systems results in light coloured crystals with the majority of protein crystals. The only drawback with this system is the impossibility to differentiate between salt crystals and protein crystals. However, any other non-crystalline material remains dark. With most crystallisation plates being manufactured out of polystyrene, this produces a highly coloured background which interferes with crystal identification. To counteract this unwanted phenomenon Greiner Bio-One developed a unique range of low birefringence plates (LBR).

In addition to providing low birefringent backgrounds (Fig. 12), the LBR plates also exhibit extremely low autofluorescence. Low autofluorescence lends itself to

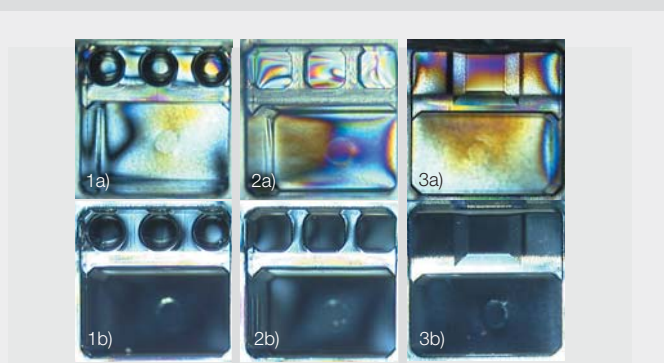
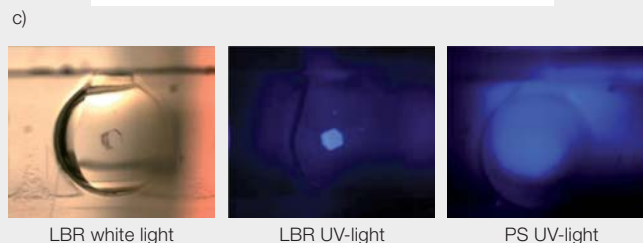
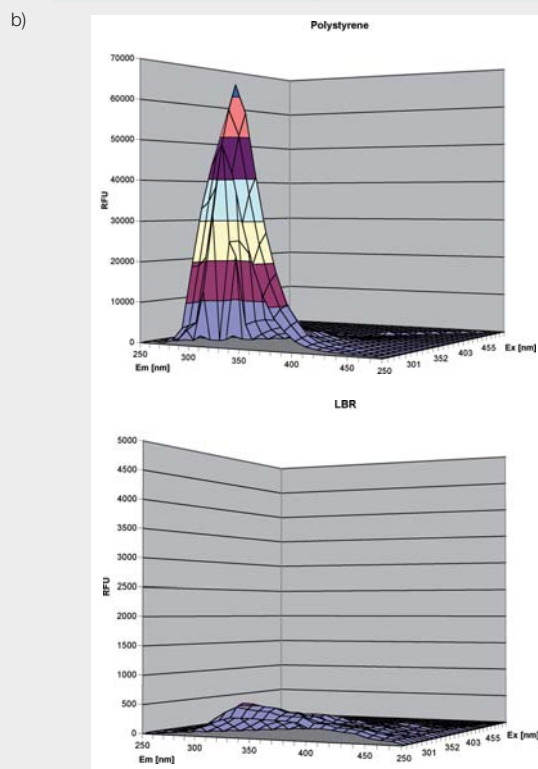
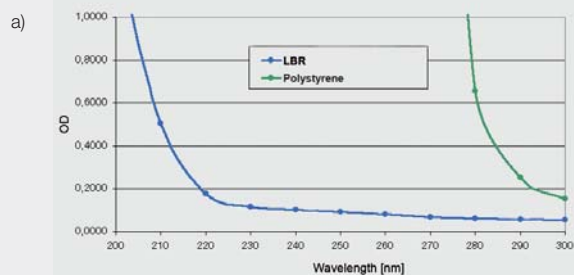


Figure 12: CrystalQuick™ plates in polarised light: (a) Standard versions with strong birefringence, (b) LBR versions with lower birefringence. (1) CrystalQuick™ RW (2) CrystalQuick™ SW (3) CrystalQuick™ LP

crystal detection via UV-light (Fig. 13) as this method relies on the amino acid tryptophan fluorescing when excited by light in the UV wavelength range. The advantage of this method is that salt crystals will not fluoresce thus reducing any false positives.



LBR white light

LBR UV-light

PS UV-light

Figure 13: Comparison of absorption in the UV-range (Fig. 13a) and of autofluorescence (Fig. 13b) of polystyrene and LBR plates. Fig. 13c shows the performance of polystyrene and LBR plates for the detection of protein crystals with UV-light. Images are courtesy of PLS-Design, Hamburg (Germany).

For an overview of our LBR plates see Appendix.

4. CrystalQuick™ Plus with Hydrophobic Surface

Approximately two thirds of drug targets today are membrane proteins. Structural analysis of membrane proteins is therefore of major interest to biopharma companies in their drug development process. Nevertheless, expression, purification and crystallisation of membrane proteins are regarded as exceptionally demanding. Even microplates have to meet exacting standards to be useful for high-throughput crystallisation of membrane proteins. CrystalQuick™ Plus plates with a hydrophobic surface are ideal for use with membrane proteins. The properties of a hydrophobic surface efficiently counteract the potential spreading of sample droplets caused by the addition of detergents to the protein solution (Fig. 14). This is also true for droplets containing surfactant precipitants such as

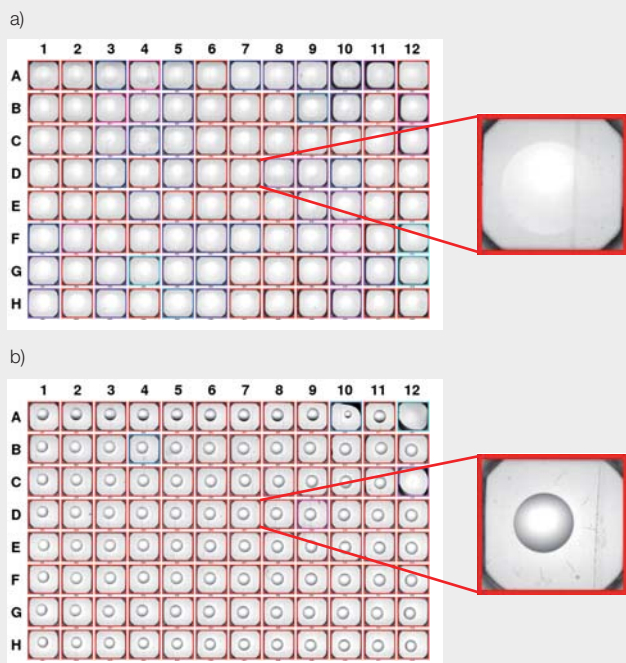
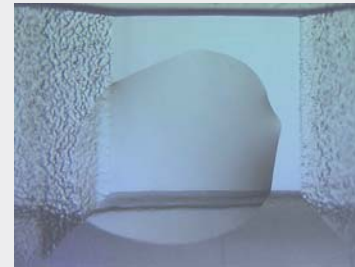


Figure 14: A standard polystyrene 96 well sitting drop flat bottom plate (Fig. 14a) and a CrystalQuick™ Plus plate (Fig. 14b) with a hydrophobic surface were compared for their performance with detergent-containing nanoliter drops. Images of 100 nl drops containing 50 mM n-Octyl-Glucoside are courtesy of Karl Harlos, The Wellcome Trust Centre for Human Genetics, Oxford (UK).



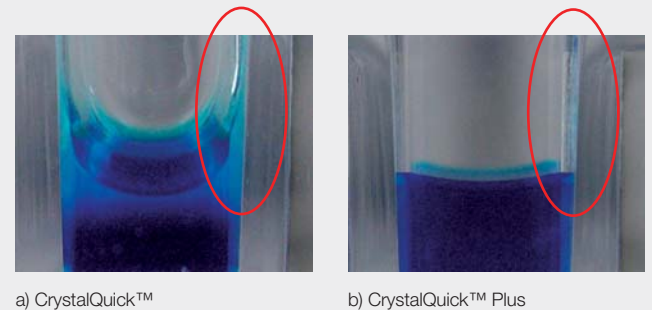
a) CrystalQuick™ LP



b) CrystalQuick™ Plus LP

Figure 15: Comparison of a CrystalQuick™ LP and a CrystalQuick™ Plus LP plate containing drops with MPD as precipitant.

MPD (Fig. 15). An additional benefit of the surface is the reduction of the meniscus of the screening solution in the reservoir. This avoids contamination via 'creeping' of the screening solution by capillary action from the reservoir into the sample well (Fig. 16).



a) CrystalQuick™

b) CrystalQuick™ Plus

Figure 16: CrystalQuick™ Plus plates reduce the risk of contamination via creeping thanks to a low meniscus in the reservoirs.

For an overview of our hydrophobic plates see Appendix.

5. APPENDIX

Products for High-Throughput Crystallography

Cat.-No.	Plates for Sitting Drop Vapour Diffusion	Qty./bag	Qty./case
609 120	96 well CrystalQuick™ RW (Round Wells)	10	40
609 101	96 well CrystalQuick™ SW (Square Wells)	10	40
609 171	96 well CrystalQuick™ LP (Low Profile)	20	80
609 820	96 well CrystalQuick™ RW LBR (low birefringent background)	10	40
609 801	96 well CrystalQuick™ SW LBR (low birefringent background)	10	40
609 871	96 well CrystalQuick™ LP LBR (low birefringent background)	20	80
609 130	96 well CrystalQuick™ Plus SW (hydrophobic surface)	10	40
609 830	96 well CrystalQuick™ Plus SW LBR (low birefringent background, hydrophobic surface)	10	40
609 180	96 well CrystalQuick™ Plus LP (hydrophobic surface)	20	80
Plates for Microbatch Crystallography under Oil			
673 170	96 well IMP@CT™ Plate, 96 conical wells, flat bottom	10	40
673 096	96 well IMP@CT™ Plate, 96 conical wells, flat bottom, black, µClear®	10	40
790 801	1536 well IMP@CT™ LBR Plate, 1536 half-conical wells, flat bottom	15	60
Lids / Sealers			
609 150	CrystalDrop™ Lid, individual well sealing with hanging drop option	5	80
609 050	CrystalDrop™ Lid, pre-greased	5	40
656 190	Low profile lid	20	200
656 101	Lid	1	100
676 070	VIEWseal™, highly transparent sealer, microencapsulated silicone adhesive	100	100
676 040	AMPLIseal™, highly transparent sealer, acrylate adhesive	100	100

Barcode Labelling

Customer-specific barcode labelling is available on request for all high-throughput crystallisation plates.

Format

We place great value on the suitability of our high-throughput crystallisation plates with automated systems. Therefore all microplates have a footprint conforming to the ANSI/SBS 1-2004 standard.

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