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Product Information

Additive Screening Kit
Product Number 78374
Store at 4°C

TECHNICAL BULLETIN

Application

The Additive Kit provides a tool for refining of promising crystallization conditions determined by our Crystallization Screening Kits. Traces of additives can manipulate interactions between sample-sample or sample-solvent interactions or structure of solvent (water). They may stabilize or increase conformity of crystals by specific interactions with biological macromolecules. Thus they may also change and improve morphology, size and diffraction quality of protein crystals.

The additive kit provides 48 different solutions of various additives, including organic solvents, salts, amino acids and other compounds. This kit provides an easy tool for rapid screening of additives. The kit includes 100 µl of each pre-formulated compound.

Quality of reagents – a key to success

The reagents within this kit are formulated using the highest purity reagents available. All solutions are sterile filtered using 0.22 micron filters. All solutions are available separately as 1 ml bottles. Larger quantities are available on request.

Storage/Stability

It is recommended that the reagents of this kit be stored at 4 °C. Storage at –20 °C will not adversely affect the kit reagents and the reagents as supplied are stable at room temperature for short-term storage. Kit reagents should not be set under ultraviolet light to protect them from microorganisms.

Handling and Procedures

The application method described below is for the sitting drop method with 10 µl drop size (e.g. in Corning Crystal*EX*™ plates, our product no Z709409). For smaller drop sizes (e.g. 2 or 4 µl in Greiner CrystalQuick™ plates, our product no. Z666130 and Z617644) smaller volumes with same ratios are used. The additives can be used for hanging drop and microbatch methods in similar manner.

1. Pipet crystallization reagent into the reservoir.

2. Pipet 5 microliters of sample onto a sitting drop post (50% of drop volume).

3. Pipet 1 microliter of Additive 1 into the sample drop (10% of drop volume).

4. Pipet 4 microliters of the crystallization reagent into the sample/additive drop. (40% of drop volume) When mixing use caution to avoid bubbles and to minimize spreading of the drop. Some drop spreading will occur due to the nature of the additive.

5. Seal the reservoir. Repeat for next additive. The sealing membrane can be pierced by standard needles used in automatic liquid handling equipment. If you need to remove the sealing membrane, first check for drops hanging at the bottom side of the membrane. If necessary, centrifuge the plate or knock it on the table to get those drops down into the reservoir.

Observation

Drops are typically observed by a stereo microscope at 10 to 100X. Record all observation by scanning every droplet on the slides.

Scan the focal plane for small crystals and record observations for all droplets. Scan the first time shortly after the screen is set up. Then for the first 5-10 days, information may be recorded daily and, thereafter, on a weekly basis. Records should follow the same scheme as without use of additives.

References

1. Michel, H. (Ed.) Crystallization of membrane proteins, CRC Press, 1991.
2. Ducruix, A., Giege, R. (Eds.), Crystallization of nucleic acids and proteins. The Practical Approach Series. Oxford Univ. Press, (1992).
3. Cudney, B. et al, Screening and optimization strategies for macromolecular crystal growth, Acta Cryst, D50, 414-423, (1994).
4. Sousa, R., Use of glycerol, polyols and other protein structure stabilizing agents in protein crystallization, Acta Cryst. (1995) D51, 271-277.
5. . Trakhanov, S. and Quiocho,F.A., Influence of divalent cations on protein crystallization Protein Science 4(9), 1914-1919 (1995).
6. Sauter, S., et al, Additives for the crystallization of proteins and nucleic acids, Journal of Crystal Growth 196, 365-375 (1999).

Precautions and Disclaimer:
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.