

# Vapor Batch Crystallization Plate

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*Solutions for Crystal Growth*

## User Guide

DI-038 & DI-040 (pg 1)

Thank you for using the Vapor Batch Crystallization Plate. This plate can be used in several different ways.

### A. Screening with modified microbatch

#### Note

The treated-hydrophilic version of the plate (DI-038 and DI-040) is recommended for screening (especially with drops below 0.5  $\mu$ l) because the drops adhere to the plate better.

1. Flood the sample area of the plate with 2.5 ml of a 50:50 mixture of silicon oil and paraffin oil (Al's Oil).
2. Dispense around 1  $\mu$ l of reagent through the oil into each sample well.
3. Dispense around 1  $\mu$ l of sample into each sample well, ensuring that it mixes with the reagent solution.
4. Replace the lid on the plate.
5. Incubate.

Trials have shown that microbatch is more efficient than vapor diffusion at picking up crystallization hits (e.g. Baldock et al., 1996; also D'Arcy et al., 2000).

### B. Screening and optimization with microbatch

#### Note

The treated-hydrophilic version of the plate (DI-038 and DI-040) is recommended for screening (especially with drops below 0.5  $\mu$ l) because the drops adhere to the plate better. The untreated-hydrophobic version of the plate (DI-039 and DI-040) is recommended for optimization because the plate reduces crystal nucleation and helps to prevent the crystals from sticking to the plastic.

Follow the same procedure as for screening, but use pure paraffin oil in step 1. Pure paraffin oil minimizes evaporation, which makes it easier to interpret experiments over time.

### C. Screening and Optimization with vapor diffusion

#### Note

The treated-hydrophilic version of the plate (DI-038 and DI-040) is recommended for screening (especially with drops below 0.5  $\mu$ l) because the drops adhere to the plate better. The untreated-hydrophobic version of the plate (DI-039 and DI-040) is recommended for optimization because the plate reduces crystal nucleation and helps to prevent the crystals from sticking to the plastic.

1. Flood the sample area of the plate with 2.5 ml of pure silicon oil.
2. Dispense around 1  $\mu$ l of crystallization reagent through the oil into each sample well.
3. Dispense around 1  $\mu$ l of sample into each sample well, ensuring that it mixes with the reagent solution.

4. Dispense around 8 ml of e.g. 0.5 M Ammonium sulfate solution into the reservoir wells around the outside of the plate. (Distribute the solution fairly evenly around the wells.)
5. Remove 1.5 ml of the silicon oil and discard.
6. Place a bead of grease around the inside of the rim of the lid. Replace the lid on the plate, ensuring that a good seal is created (or seal with tape).
7. Incubate.
8. If no crystals grow, or if precipitation is in general very light, try increasing the concentration of the reservoir solution in steps.

#### Note

- a. The silicon oil used in microbatch screening allows slow evaporation. This gives a result which is similar to vapor diffusion in that it scans through many crystallization conditions. The proportion of silicon oil can be adjusted – using more silicon increases evaporation, using less reduces it.
- b. If you use less than 1  $\mu$ l + 1  $\mu$ l drops in microbatch experiments, reduce the proportion of silicon oil.
- c. If you dispense automatically, accuracy can be improved by dispensing the sample and reagent solution first, then covering with 10  $\mu$ l of paraffin oil straight away.
- d. If you get crystals of salt, try increasing the volume of sample or decreasing the volume of reagent.
- e. For maximum crystal nucleation, do not stir the droplet. To reduce nucleation, stir.

#### References

P.E.M. Baldock, V. Mills, P.D. Shaw Stewart. A comparison of microbatch and vapour diffusion for initial screening of crystallization conditions. *Journal of Crystal Growth*. 168 (1996), pp 170-174.

A. D'Arcy, G.E. Dale, M. Stihle, B. D'Arcy. Results reported at the 8th International Conference on the Crystallization of Biological Macromolecules, May 18, 2000. When the group screened 10 proteins with both methods (using robots), "modified microbatch" - i.e. microbatch using the silicon/paraffin mixture - yielded 104 hits, compared to 62 hits with vapor diffusion.

#### Technical Support

Inquiries regarding the Vapor Batch Plates and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 5:00 p.m. USA Pacific Standard Time.

#### Related Products

**DI-038** Vapor Batch 96 Well Treated, Hydrophilic Plate - 10 plate sampler  
**DI-040** Vapor Batch 96 Well Treated, Hydrophilic Plate - 80 plate case

**DI-039** Vapor Batch 96 Well Untreated, Hydrophobic Plate - 10 plate sampler  
**DI-041** Vapor Batch 96 Well Untreated, Hydrophobic Plate - 80 plate case

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DI-038 & DI-040 (pg 2)

## Related Products

HR3-411	Paraffin Oil - 250 milliliters
HR3-421	Paraffin Oil - 1 liter
HR3-413	Al's Oil (1:1 Paraffin:Silicon) - 250 milliliters
HR3-415	Silicon Oil - 250 milliliters
HR3-423	Silicon Oil - 1 liter
HR3-417	Crystal Oil Combination Pack - one of each oil above
HR3-510	Vacuum Grease, 150 gram tube
HR3-508	DC 7 Release Compound Grease, 150 gram tube
HR3-504	Grease Kit – one each (grease not included)
HR3-506	Grease Kit – five pack (grease not included)
HR2-130	Crystal Screen HT
HR2-134	Index HT Screen
HR2-136	SaltRx HT Screen
HR2-137	MembFac HT Screen
HR2-138	Additive HT Screen
HR2-139	PEG/Ion HT Screen
HR2-247	Grid Screen Salt HT Screen

Hampton Research  
34 Journey  
Aliso Viejo, CA 92656-3317 U.S.A.  
Tel: (949) 425-1321 • Fax: (949) 425-1611  
Technical Support e-mail: [tech@hrmail.com](mailto:tech@hrmail.com)  
Website: [www.hamptonresearch.com](http://www.hamptonresearch.com)