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Cation crystallisation

The Small Molecule Anion screen MD1-125

The Small Molecule Anion screen is a crystallisation screen for organic salts, active pharmaceutical ingredients, coordination complexes and peptides.

The Small Molecule Anion screen is uniquely aimed at bringing the power of 96-well high-throughput crystallisation screening to organic salts and active pharmaceutical ingredients.

MD1-125 is presented as 96 x 1 mL conditions in a deep-well block.

Simple, practical crystallization screening for cations:

- Increase your lab throughput with rapid screening for:
  - Organic salts soluble in water down to 2 mg/ml
  - Active pharmaceutical ingredients (API)
  - Coordination complexes
  - Peptides
  - Proteins with pI > 7
- Increase chance of crystallization with 77 different anions screened.
- Suitable for manual as well as robotic set-up: no specialist equipment required.
- Perfect for most crystallization protocols: works equally well for under-oil and vapor diffusion.

Bring the power of high-throughput crystallisation screening to cations.

For the first time ever, you can have the speed, convenience, and power of macromolecular high-throughput screening techniques for cation crystallization with our new Small Molecule Anion Screen. Developed by Dr Bernhard Spingler of the University of Zurich, and available exclusively from Molecular Dimensions, this high-throughput sparse matrix screen provides everything required for 96-well under-oil screening for water soluble organic salts, cation complexes, and active pharmaceutical ingredients. The screen covers a broad region of chemical space, utilizing 77 different anions, so it can also be used as a coarse screen for macromolecules with an isoelectric point > 7.

Under-oil Microbatch or Vapour Diffusion?

This screen is suitable for use with vapour diffusion or micro-batch under-oil techniques. Successful crystallisations have been achieved with both techniques. However, Babor *et al* (2019) shows that the screen produces higher hit rates with microbatch under-oil technique.

Complete kit and protocol provided.

Many researchers who may potentially interested in this screen will not have carried out crystallisations in a 96-well format previously, and may not have ready access to all the required equipment. For this reason, we recommend that users should start with under-oil crystallisation. This crystallisation technique is simple to carry out without robotic assistance, and is a form of microbatch crystallisation with which the user may already be familiar. The Small Molecule Anion kit is provided with full instructions and everything required for an under-oil crystallisation, including plates, lids to prevent spillage and appropriate oils. This datasheet also includes full instructions for how to set up an under-oil crystallisation. The only additional material that is required are variable volume pipettes in 0.1-10 and 1-1000 µL and appropriate disposable tips.

Screen developer Dr Bernhard Spingler of Zurich University.

(+)-Ephedrinium iodide crystals grown using the new Small Molecule Anion Screen with the under-oil technique, courtesy of Dr Bernhard Spingler.

References.

Nievergelt, P. P., Babor, M., Čejka, J.; Spingler, B. (2018) *Chem. Sci.* **9**, 3716-3722.

Babor, M.; Nievergelt, P. P.; Čejka, J.; Zvoníček, V.; Spingler, B. (2019) *IUCrJ* **6**, 145-151.

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Protocol for under oil crystallization technique:

Starting Materials

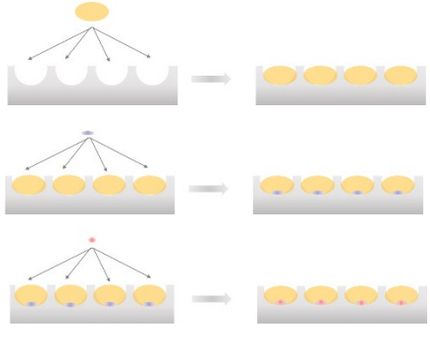
Small Molecule Anion Under-oil crystallisation complete kit (MD1-125-UO) **or**:

- MD1-125 Small Molecule Anion (MD1-125)
- 96-well Microbatch under-oil plate, such as MD11-41-UVP from Molecular Dimensions
- silicone oil with a viscosity of 50 or 5 cSt (for example MD2-08 from Molecular Dimensions)

In all cases, for an efficient setup you will also require ideally two eight-channel pipettes that can pipette 5 and 100 µL, a single channel pipette may also be used, but will be slower.

Protocol

1. Pipette 20 µL silicone oil into each of the 96 wells of the microbatch underoil plate.
2. Pipette 5 µL of your 90% saturated aqueous solution with the cation of interest at the bottom of all 96 wells already containing silicone oil.
3. Pipette 5 µL of each of the 96 Small Molecule Anion Screen conditions at the bottom of the corresponding well. You must make sure that the two aqueous drops within the silicone oil merge to give one single drop
4. Check the drops for crystal growth every day.
5. Once the crystal growth in a particular well has stopped, remove the crystals and measure them immediately (if possible). As the drops continue to concentrate, the starting salt or other minor components might crystallize



6. If nothing happens in a drop, scratch the bottom of the well with a dissecting needle, to trigger nucleation.

IMPORTANT INFORMATION

- You can increase the volume ratios of Small Molecule Anion screen versus cation solution, which will favour the anion exchange
- The total volume of the two aqueous drops combined should not be smaller than 10 µL.
- You should check all drops at the end of pipetting with help of a transmitted-light microscope, to ensure they have merged to one single drop per well.

Protocol for vapour diffusion crystallization technique:

Starting Materials / Requirements:

- Small Molecule Anion Screen (MD1-125)
- MRC vapour diffusion plate (for example 96-well 2-drop, MD11-00-100 or 48-well MAXI optimisation, MD11-004-100
- Plate seals (for example MD6-01S ClearVue Seals)

Protocol

1. Add 50-200 µL of each of the 96 Small Molecule Anion Screen conditions into the corresponding large reservoir well on your plate of choice.
2. To set-up protein drops in the sample wells of the plate: using a new pipette tip place 1 µL of reservoir buffer from the reservoir well into the sample well, then place 1 µL of your 90% saturated aqueous solution with the cation of interest on top of the reservoir drop.
3. Seal the plate or rows with a plate seal. The MD6-01S are ClearVue seals are pressure-burst seals and pressure needs to be applied to the seal in order for the adhesive to seal them properly (this is done with the seal applicator or a ruler). These sheets do not feel sticky when the backing is removed because of this.
4. Leave in an incubator or temperature-controlled room at your desired incubation temperature.

Alternatively:

If you have access to a pipetting robot, this may be utilised for vapour diffusion crystallisation set up and normally includes all the needed materials except your sample of interest and the Small Molecule Anion Screen

Formulation Notes.

The Small Molecule Anion Screen reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 µm filters. No preservatives are added.

Final pH may vary from that specified on the datasheet. Molecular Dimensions will be happy to discuss the precise formulation of individual reagents.

Individual reagents and stock solutions for optimization are available from Molecular Dimensions.

Enquiries regarding the Small Molecule Anion Screen formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

Contact and product details can be found at www.moleculardimensions.com.

Manufacturer's safety data sheets are available from our website.

RE-ORDERING INFORMATION

	Pack Size	Description
MD1-125-UO	1 kit	Small Molecule Anion Screen kit: 1 x MD1-125 Small Molecule Anion Screen, 5 x Swissci microbatch plates (MD11-41-UVP), 1 x Silicone 5cSt oil (MD2-08), complete instructions for setting up under-oil crystallization by hand or robot.
MD1-125	96 x 1 mL	Small Molecule Anion Screen
MD2-08	50 mL	Silicone 5cSt Oil
MD11-41-UVP(-10)	100 (10) plates	UV and circular polarized light transparent Swissci microbatch plates.

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The Small Molecule Anion Screen MD1–125 Conditions A1–H12

Screen ID	Well #	Conc.	Unit	Precipitant
1-1	A1	3.00	M	Sodium chloride
1-2	A2	1.50	M	Sodium chloride
1-3	A3	4.00	M	Sodium bromide
1-4	A4	2.00	M	Sodium bromide
1-5	A5	5.30	M	Sodium iodide
1-6	A6	2.50	M	Sodium iodide
1-7	A7	1.25	M	Sodium iodide
1-8	A8	7.30	M	Potassium thiocyanate
1-9	A9	3.50	M	Potassium thiocyanate
1-10	A10	1.75	M	Potassium thiocyanate
1-11	A11	0.70	M	Sodium dicyanamide
1-12	A12	4.00	M	Sodium tetrafluoroborate
1-13	B1	2.00	M	Sodium tetrafluoroborate
1-14	B2	0.24	M	Potassium hexafluorophosphate
1-15	B3	0.40	M	Sodium tetraphenylborate
1-16	B4	0.20	M	Sodium tetraphenylborate
1-17	B5	1.00	M	Disodium sulfate
1-18	B6	3.60	M	Sodium methanesulfonate
1-19	B7	1.80	M	Sodium methanesulfonate
1-20	B8	0.80	M	Sodium triflate
1-21	B9	2.20	M	Sodium isethionate
1-22	B10	1.10	M	Sodium isethionate
1-23	B11	2.28	M	Sodium (+/-)-camphorsulfonate
1-24	B12	0.98	M	Sodium benzenesulfonate
1-25	C1	0.42	M	Sodium 3-nitrobenzensulfonate
1-26	C2	0.15	M	Sodium *p*-toluenesulfonate
1-27	C3	0.35	M	Sodium 1-naphthalensulfonate
1-28	C4	0.13	M	Sodium 2-naphthalensulfonate
1-29	C5	0.085	M	Disodium 2,6-naphthalenedisulfonate
1-30	C6	4.60	M	Sodium nitrate
1-31	C7	2.30	M	Sodium nitrate
1-32	C8	1.80	M	Sodium benzoate
1-33	C9	2.20	M	Sodium salicylate
1-34	C10	1.10	M	Sodium salicylate
1-35	C11	1.50	M	Sodium 4-aminosalicylate dihydrate
1-36	C12	1.30	M	Sodium meta-hydroxybenzoate
1-37	D1	2.96	M	Sodium nicotinate
1-38	D2	1.48	M	Sodium nicotinate
1-39	D3	0.27	M	Potassium hydrogen phthalate
1-40	D4	1.40	M	Disodium isophthalate
1-41	D5	0.060	M	Disodium terephthalate
1-42	D6	0.050	M	Disodium pamoate
1-43	D7	6.00	M	Sodium formate
1-44	D8	3.00	M	Sodium formate
1-45	D9	2.60	M	Sodium acetate
1-46	D10	2.40	M	Sodium trifluoroacetate
1-47	D11	1.70	M	Sodium 2-phenylpropionate
1-48	D12	0.25	M	Sodium DL-mandelate

Screen ID	Well #	Conc.	Unit	Precipitant
2-1	E1	0.25	M	Sodium D-mandelate
2-2	E2	0.25	M	Sodium L-mandelate
2-3	E3	0.43	M	Sodium 1-naphthalenacetate
2-4	E4	0.33	M	Sodium diphenylacetate
2-5	E5	2.28	M	Sodium *N*-acetylglycinate
2-6	E6	1.46	M	Sodium hippurate
2-7	E7	4.96	M	Sodium pyrrolidone carboxylate
2-8	E8	5.20	M	Sodium propionate
2-9	E9	2.60	M	Sodium propionate
2-10	E10	3.42	M	Sodium DL-lactate
2-11	E11	3.42	M	Sodium L-lactate
2-12	E12	3.00	M	Sodium pyruvate
2-13	F1	1.50	M	Sodium pyruvate
2-14	F2	3.19	M	Sodium valerate
2-15	F3	2.70	M	Sodium hexanoate
2-16	F4	4.20	M	Sodium 2-ethylhexanoate
2-17	F5	2.10	M	Sodium 2-ethylhexanoate
2-18	F6	1.10	M	Potassium gluconate
2-19	F7	1.56	M	Sodium octanoate
2-20	F8	0.60	M	Sodium hydrogen carbonate
2-21	F9	1.00	M	Disodium carbonate
2-22	F10	0.14	M	Disodium oxalate
2-23	F11	2.97	M	Disodium malonate
2-24	F12	1.13	M	Disodium succinate
2-25	G1	0.66	M	Disodium maleate
2-26	G2	0.73	M	Disodium fumarate
2-27	G3	2.27	M	Disodium DL-malate
2-28	G4	2.92	M	Disodium L-malate
2-29	G5	1.40	M	Sodium potassium L-tartrate
2-30	G6	0.55	M	Potassium sodium DL-tartrate tetrahydrate
2-31	G7	1.00	M	Disodium L-tartrate
2-32	G8	0.26	M	Disodium (+)-O,O'-dibenzoyl-D-tartrate
2-33	G9	0.054	M	Potassium antimony tartrate
2-34	G10	1.63	M	Disodium N-acetylglutamate
2-35	G11	1.19	M	Disodium adipate
2-36	G12	0.050	M	Potassium D-saccharate
2-37	H1	0.90	M	Trisodium citrate dihydrate
2-38	H2	0.25	M	Potassium DL-aspartate
2-39	H3	0.25	M	Sodium L-aspartate
2-40	H4	2.00	M	Sodium L-glutamate
2-41	H5	1.00	M	Sodium L-glutamate
2-42	H6	0.011	M	Sodium diethyldithiocarbamate
2-43	H7	1.57	M	Sodium saccharine
2-44	H8	0.43	M	Disodium hydrogen phosphate
2-45	H9	4.00	M	Sodium dihydrogen phosphate
2-46	H10	2.00	M	Sodium dihydrogen phosphate
2-47	H11	0.93	M	Disodium citrate
2-48	H12	0.36	M	Trisodium phosphate (dodecahydrate)

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