Additive screening protocol 1 (setting up crystallization droplets in a 96-well crystallization plate pre-filled with additive screen)

1. First, prepare the condition with initial concentrations of reagents increased by 10% (min. vol. 15 mL when transferring the condition from a container onto the additive screen with the liquid handler).

2. Ensure that the liquid handler is ready to operate. Open the program 'Add screen to additives' for a single plate (enter volume in reservoirs: '72 µL'). *Prompts, figures and a tip-management system (12 x 1,000 µL tips required) help with making sure the robot is ready to operate according to the selections.*

3. Retrieve the additive screen from the -20 °C incubator and remove its aluminum seal immediately (use the plate holder), then place the plate on the deck of the liquid handler. *The program operates according to a portrait layout (the plate is placed with the A1-corner located in the front-left of the carrier).* Also place also the container filled with the condition. Run the program.

4. Once the reservoirs of the plate have been filled, place it onto the microplate shaker and run the program. Rinse the container of condition with deionized water and 20 % ethanol for reuse.

5. **Set up the droplets on the nanoliter dispenser**. First, unseal the crystallization plate (use the plate holder). Then, place the plate and the 8-well protein strip in the first position of the strip-holder block. *The 'Setup' tab on the controlling software displays the actual positions of each component on the deck*. Load each well of the strip with protein sample according to the drop size required (**Table 1**). Run the program to prepare droplets.

6. Upon completion of the program, remove the plate from the deck and seal it immediately (use the plate sealer, 3-inch wide adhesive tape). Discard the strip in the appropriate bin.

7. Assess the size, shape, and centering of the droplets under the microscope before storage.

Additive screening protocol 2 (setting up crystallization droplets in a 96-well crystallization plate with a re-usable additive screen)

1. First, prepare the additive screen: leave the corresponding frozen 96-well cell culture plate to thaw at room temperature for 40 min. Then, centrifuge the additive screen at 1,000 x g for 2 min.

2. Prepare the condition (min. vol. 15 mL when transferring the condition from a container onto the additive screen with the liquid handler).

3. Fill the reservoirs of a 96-well crystallization plate with the condition. On the liquid-handler,

proceed the same way as protocol 1, step 2, but enter '80 µL' for the volume in reservoirs.

4. Set up the droplets on the nanoliter dispenser. Proceed the same way as protocol 1, steps 2-6, but with 3 components on the deck instead of 2 (the plate containing the additive screen, along with the crystallization plate and the 8-well protein strip in the first position of the strip-holder block, **Table 1**).

5. Seal the cell culture plate containing the screen with an aluminum sheet and place it back in the -20 °C incubator.

6. Assess the size, shape, and centering of the droplets under the microscope before storage.

Table 1: Programs available on the microsyringe-based nanoliter dispenser for additive screening. The required volume of sample in each well of the 8-well strip is calculated as follows: dead volume + 12 x drop size. There are 2 types of strips (2 µL and 5 µL). The dead volumes are 0.8 µL for the 2 µL wells (which can actually contain 3.2 µL max.) and 1.4 µL for the 5 µL wells (7.5 µL max.). The total volume of protein required is: 8 x volume in well of the strip (round-up value). The dead volume of the V-shaped wells of the 96-well cell culture plate is 2.5 µL. The required number of microsyringes varies according to the selected program (8 + 96 = 104; or 8 + 2 x 96 = 200).

Protocol		Drop size (nL)			Strip type	Sample vol. (µL)			
Type	Source of additives	Protein	Condition	Additive		In each well (strip)	Total	No. of tips	Duration
1	MRC plate	100	100	0	2 µL	2	16	104	2 min
1	MRC plate	200	200	0	5 µL	3.8	31	104	2min 10 sec
2	Costar plate	200	200	100	5 µL	3.8	31	200	3min 45 sec
2	Costar plate	500	500	100	5 µL	7.4	60	200	4min 15 sec