

## Crystallization Solution E Protocol

### Preparation of a Crystallization Solution.

- Created from the acidic and basic form of one chemical (e.g., sodium malonate) both at the same concentration
- The pH is adjusted by the addition of one component to the other

### Step 1: Identification of the crystallization solution characteristics.

The required component concentration(s) can be found in the production report (see example in Table 1).

### Step 2: Calculation of the amount of each chemical required to prepare 1 liter of stock solution.

Amount required in g = molarity (M) x FW x liters of solution being prepared.

Using the example in Table 1:

Amount of chemical X required in g = 3.4 x 75.00 x 1 = 255.00 g

Amount of chemical Y required in g = 3.4 x 87.00 x 1 = 295.80 g

### Step 3: Crystallization solution preparation (1 liter).

1. Weigh the required amount of each chemical into separate beakers.
2. Add water to 95% of the final volume (~950 ml) and stir thoroughly until completely dissolved.
3. Add water to give a final volume of 1 liter.
4. Suspend a pH meter in the solution of the acid form of the chemical, and slowly add the base form to bring the pH near to the desired value.
5. Store the crystallization solution at room temperature for 24 h\*.
6. Adjust the pH using the base form to the desired value.
7. Filter the crystallization solution using a 0.22 µm filter.
8. Transfer the solution to the desired format (e.g., 24- or 96-well plate) in a sterile environment.

\* Since the acid/base reaction is usually exothermic, this step will bring the solution back to room temperature.

**Table 1. Identification of the Crystallization Solution characteristics.**

Formulation	Chemical FW
3.4 M Chemical X (acid form)	75.00
3.4 M Chemical Y (base form)	87.00
Final pH targeted: 6.0	

The value of a chemical's formula weight (FW) is the sum of the atomic weights of the atoms found in one formula unit (including its hydration state) of an ionic compound. For other information about each chemical, please refer to the production report specific for each solution.

### Things to remember:

- All chemical information can be found in the production report for each solution.

- Amount of chemical required for each solution is calculated thus:

If concentration is given as X M:

$$\text{Amount in grams} = X \times \text{FW} \times \text{FV (liters)}$$

If the concentration is given as X% v/v:

$$\text{Volume required} = X \text{ ml per } 100 \text{ ml}$$

If the concentration is X% w/v:

$$\text{Amount in grams required} = X \text{ g}/100 \text{ ml}$$

### Units Definition

M: Molarity of chemical

FV: Final volume of solution

FW: Formula weight of chemical