

Auxin/IAA Preparation

A. Make a 20% w/v stock solution of IAA in 95% ETOH.

Example:

In a 1.5ml microtube, dissolve 200mg of IAA in 1ml 95% ETOH.

B. Make substock solutions as needed. In our case, we needed 60μM, 400μM, and 4000μM substock solutions of IAA.

Example:

1) Determine amount of 20% w/v stock IAA solution needed to make the necessary substock solutions.

$$\frac{60\mu\text{mol}}{1\text{L}} \times 250\text{ml} \times \frac{1\text{L}}{10^3\text{ml}} \times \frac{175.18\text{g}}{1\text{mol}} \times \frac{1\text{mol}}{10^6\mu\text{mol}} \times \frac{1\text{ml}}{0.2\text{g}} = 0.0131\text{ml}$$

$$\frac{400\mu\text{mol}}{1\text{L}} \times 100\text{ml} \times \frac{1\text{L}}{10^3\text{ml}} \times \frac{175.18\text{g}}{1\text{mol}} \times \frac{1\text{mol}}{10^6\mu\text{mol}} \times \frac{1\text{ml}}{0.2\text{g}} = 0.035\text{ml}$$

$$\frac{4000\mu\text{mol}}{1\text{L}} \times 25\text{ml} \times \frac{1\text{L}}{10^3\text{ml}} \times \frac{175.18\text{g}}{1\text{mol}} \times \frac{1\text{mol}}{10^6\mu\text{mol}} \times \frac{1\text{ml}}{0.2\text{g}} = 0.0876\text{ml}$$

2) Add the correct amount of stock IAA to the appropriate sized volumetric flask. Fill the flask to volume with nanopure-filtered water and mix thoroughly.

C. Determine the amount of substock IAA solution needed to add to the media. In our case, we wanted 0, 0.01, 0.1, 1, 10, and 30 μM IAA in our final media (volume of 200ml).

Example: $\frac{0.01\mu\text{M} \times 200\text{ml}}{60\mu\text{M}} = 0.0333\text{ml}$ of 60μM IAA

D. Prepare the media without IAA and autoclave with a stir bar. After autoclaving, when the media is ~50°C, add the correct amount of IAA to each flask and stir gently on a magnetic stirrer to completely mix in the IAA. Pour the plates.

E. Store the IAA stock and substock solutions in the freezer.

Prepared by: Colleen van Pelt

Note: All protocols are provided on an as-is basis. Please check all calculations for accuracy.