

TD-P Revision 3.0

Protocol

Creation Date: 7/18/2014 Revision Date: 6/25/2020

Preparation of Indole-3-Acetic Acid (IAA) Stock Solution IAA Stock Solution (10 mg/mL)

Introduction

Indole-3-Acetic Acid (IAA) is poorly soluble in H_2O or aqueous buffers, unless the pH of the solvent is alkaline. 1N NaOH is the preferable solvent to dissolve IAA. The use of this solvent minimizes the addition of organic solvents into the final experiment protocol.

IAA is also soluble in some organic solvents at the following ratios:

- Ethanol (up to 140 mg/ml)
- DMSO (1.08 g/ml), however the solution is viscous.
- DMF (1.02 g/ml)

Materials

- Indole-3-acetic acid (IAA) (<u>GoldBio Cat # I-110</u> [CAS 87-51-4, mw = 175.18])
- 1N NaOH

Method

1. Dissolve 0.5 g of IAA in 3 ml of 1N NaOH.

Note: This solution is very close to saturation. To dissolve the IAA completely, add additional 0.25 ml of 1N NaOH. Heating should not be necessary to dissolve the IAA.

- 2. Once completely dissolved, add the solution dropwise and stir. While stirring, bring to volume with any of the following solvents:
 - Final solution unbuffered:

Add the IAA/NaOH solution to 45 ml of H_2O . Bring final volume to 50 ml with H_2O . Your final pH will be very alkaline and unbuffered.

- Buffered IAA:

Add the IAA/NaOH solution to 45 ml of 50-100mM Sodium Phosphate buffer, pH 7.2. Bring final volume to 50 ml with 50-100mM Sodium Phosphate buffer, pH 7.2. The final pH will range from about pH 7.4 to pH 7.2, respectively.



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- IAA in PBS: Add the IAA/NaOH solution to 45 ml 1x PBS. Bring final volume to 50 ml with 1x PBS buffer. The pH of this solution will be very alkaline since 1x PBS has low buffering capacity.
- IAA in PBS with extra buffer:
 Add the IAA/NaOH solution to 40 ml 1x PBS. Then add 5 ml of 0.5M Sodium
 Phosphate buffer pH 7.2 to neutralize the 1N NaOH and reduce the pH to 7.2 -7.4.
 Bring final volume to 50 ml with 1x PBS.
- 3. Sterilize by autoclaving or filtering through a 0.2 μm filter.

Storage

There are conflicting reports in the literature about long term stability of aqueous solutions of IAA. We suggest to empirically determine the IAA activity of solutions stored at -20°C.

Reference

Yamakawa, T., Kurahashi, O., Ishida, K., Kato, S., Kodama, T., & Minoda, Y. (1979). Stability of indole-3-acetic acid to autoclaving, aeration and light illumination. Agricultural and Biological Chemistry, 43(4), 879-880.