

SOP-P029

Preparation of Stock α -MEM Medium

Objective: To prepare one liter of stock α -MEM medium for maintaining C5 CHO cells for culture and assay

Procedure:

1. In a clean beaker measure out 900 ml Millipore filtered water at room temperature.
2. Add a stir bar to the beaker.
3. While stirring gently on a magnetic stir plate, add the contents of one 1 L bottle powdered Minimum Essential Medium Eagle, alpha modification (Sigma #M-0894).
4. Rinse the original container with a small amount of Millipore filtered water to remove remaining powder and add the rinse to the solution in the beaker.
5. Stir until the powder is dissolved. Do not heat the mixture.
6. Add to the solution 2.2 g sodium bicarbonate (NaHCO₃) and continue stirring until dissolved.
- 7a. Continue to stir and determine the pH of the solution using an Orion Research EA920 Expandable Ion Analyzer (refer to SOP#-P024 for Determination of pH Using an Orion Research EA920 Expandable Ion Analyzer). The analyzer is located in the Hansen building, room B050.
- 7b. Adjust the pH of the solution to 7.2. Using a 5.25" Pasteur pipette fitted with a rubber pipette bulb, add drop wise 1 N hydrochloric acid (HCl) if the pH is higher than 7.4, or 1 N sodium hydroxide (NaOH) if the pH is lower than 7.4, until the correct pH is obtained.
8. Remove the stir bar and pour the medium into a graduated cylinder.
9. Add Millipore filtered water until the volume in the cylinder is 1000 ml.
10. In a sterile environment such as a laminar flow hood, immediately sterilize the medium by filtration through a sterile filter with a porosity of 0.2 μ m.
11. Aseptically dispense the medium into sterile containers such as autoclaved glass bottles with screw caps.
12. Label the containers with content information, date, and your initials.
13. Store the medium refrigerated at 0-5°C. Expiration date is approximately six months after date of preparation. Deterioration can be recognized by any of the following: 1) pH change, 2) precipitation, 3) cloudy appearance, or 4) color change.

Created by: Gretchen Lawler

Verified by: _____ **Date:** _____

Print Name

Sign Name