SOP-P009

Preparation of Working Hanks Balanced Salt Solution (HBSS) With Calcium, Magnesium, and 0.1% Bovine Serum Albumin (BSA)

Objective: To prepare a buffer for washing, suspending, and diluting cells.

Procedure:

1. Measure out 80 ml millipore filtered water in a graduated cylinder.

2. Pour the water into a clean Erlenmeyer flask containing a stir bar.

3. While stirring gently using a magnetic stirplate, add the following to the flask:
   10 ml 10x HBSS (refer to SOP#-P001 for Preparation of 10x Hanks Balanced Salt Solution)
   2.75 ml Tris 1.0 M (refer to SOP#-P028 for Preparation of Tris 1.0 M)
   170 µl calcium chloride 1.1 M ($CaCl_2$) (refer to SOP#-P003 for Preparation of Calcium Chloride 1.1 M)
   200 µl magnesium sulfate 0.4 M ($MgSO_4$) (refer to SOP#-P018 for Preparation of Magnesium Sulfate 0.4 M)
   220 mg glucose
   100 mg bovine serum albumin

4. Stir until dissolved.

5a. Continue to stir and determine the pH of the solution using an Orion Research EA 920 Expandable ionAnalyzer (refer to SOP#-P024 for Determination of pH Using an Orion Research EA 920 Expandable ionAnalyzer). The analyzer is located in the Hansen building, room B050.

5b. Adjust the pH of the solution to 7.4. Using a 5.25" Pasteur pipet fitted with a rubber pipet bulb, add dropwise 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.

6. Remove the stir bar and pour the solution into a graduated cylinder.

7. Add millipore filtered water to the solution until the volume reaches 100 ml.

8. Return the Hanks balanced salt solution to the flask and cover the top with a square of aluminum foil or parafilm.

9. Label the flask with content information, date, and your initials.

10. Keep at 0-5°C and discard unused portion at the end of the day.

Created by: Gretchen Lawler

Verified by:_____ Date:_____ _____

Print Name Sign Name