

SOP-P013

Preparation of Working Hanks Balanced Salt Solution (HBSS) Without Calcium, Magnesium, and with 1% Bovine Serum Albumin (BSA)

Objective: To prepare a calcium/magnesium-free 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 stir plate, add the following to the flask:
 - 10 ml 10 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)
 - 220 mg glucose
 - 1.00 g 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 ion Analyzer (refer to SOP#-P024 for Determination of pH Using an Orion Research EA 920 Expandable ion Analyzer). 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 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.
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