

### **KREBS-HENSELEIT BUFFER**

With 2000 mg/L Glucose, Without Calcium Chloride and Sodium Bicarbonate Product Number **K3753** 

## **Product Description**

Krebs-Henseleit buffer was developed in the early 1930's by Hans Krebs and Kurt Henseleit. This modification of Ringer's solution was used to maintain liver tissue during experiments that led Krebs to postulate the urea cycle. The formula offered by Sigma has been modified by the addition of 2 g/L of glucose as an energy source for cell maintenance, and by the omission of calcium chloride.

Components	<u>g/L</u>
D-Glucose	2.0
Magnesium Sulfate [Anhydrous]	0.141
Potassium Phosphate Monobasic	0.16
Potassium Chloride	0.35
Sodium Chloride	6.9

## **Precautions and Disclaimer**

REAGENT

For R&D use only. Not for drug, household or other uses.

### **Preparation Instructions**

Powdered salts are hygroscopic and should be protected from moisture. The entire contents of each package should be used immediately after opening. Preparing a concentrated salt solution is not recommended as precipitates may form. Supplements can be added prior to filtration or introduced aseptically to sterile salt solution.

- 1. Measure out 90% of final required volume of water. Water temperature should be 15-20 °C.
- While gently stirring the water, add the powdered medium. Stir until dissolved. Do NOT heat.
- 3. Rinse original package with a small amount of water to remove all traces of powder. Add to solution in step 2.
- 4. To the solution in step 3, add 0.373 g of calcium chloride dihydrate for each liter of solution being prepared. Stir until dissolved.
- 5. To the solution in step 4, add 2.1 g sodium bicarbonate or 28 ml of sodium bicarbonate solution [7.5% w/v] for each liter being prepared. Stir until dissolved.

- While stirring, adjust the pH of the medium to 0.1-0.3 pH units below the desired pH since it may rise during filtration. The use of 1N HCl or 1N NaOH is recommended.
- Add additional water to bring the solution to final volume.
- 8. Sterilize immediately by filtration using a membrane with a porosity of 0.22 microns.
- 9. Aseptically dispense medium into sterile container.

# Storage and Stability

Store the dry powdered salts at 2-8 °C under dry conditions and liquid medium at 2-8 °C in the dark. Deterioration of the powdered medium may be recognized by any or all of the following: [1] color change, [2] granulation/clumping, [3] insolubility. Deterioration of the liquid medium may be recognized by any or all of the following: [1] pH change, [2] precipitate or particulates, [3] cloudy appearance [4] color change. The nature of supplements added may affect storage conditions and shelf life of the medium. Product label bears expiration date.

### Procedure

Materials Required but Not Provided:
Water for tissue culture [W3500]
Sodium Bicarbonate [S5761] or
Sodium Bicarbonate Solution, 7.5% [S8761]
Calcium Chloride, Dihydrate [C7902]
1N Hydrochloric Acid [H9892]
1N Sodium Hydroxide [S2770]
Medium additives as required

# Reference

Krebs, H. A. and Henseleit, K. (1932).
 Untersuchungen über die Harnstoffbildung im Tierkörper. Hoppe-Seyler's Zeitschrift für Physiol. Chemie. 210, 33-66.

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