3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

EARLE'S BALANCED SALTS [EBSS]
Without Sodium Bicarbonate

Product Number **E6132**
Storage Temperature 2-8 °C

Product Description

The search for a synthetic medium to replace serum for maintaining cells in vitro began in the late nineteenth century and continues to this day. Ringer, Locks and Tyrode substituted physiological salt solutions augmented with glucose for serum and thereby laid the foundation for the development of defined media.

As biochemical and analytical techniques have improved, more of the components in serum such as vitamins, hormones, and amino acids have been identified and incorporated into physiological salt solutions, reducing, and in some cases eliminating the concentrations of animal sera required as a medium supplement.

Although there have been many modifications to the original formulas in efforts to produce fully defined media, salt solutions still play an important role in tissue culture. A salt solution's basic function, to maintain the pH and osmotic balance in the medium and to provide the cells with water and essential inorganic ions, is as valuable today as when it was first developed a century ago.

EARLE'S BALANCED SALTS [EBSS], Product No. E6132 is one of the balanced salts available from Sigma. The selection of a balanced salt is strongly influenced by 1] type of cell, 2] type of culture [monolayer, suspension, clonal] and 3] degree of chemical definition necessary. It is important to review the literature for recommendations concerning medium, supplementation and physiological parameters required for a specific cell line.

Components	g/L
Calcium Chloride•2 H_2O	0.265
Magnesium Sulfate (anhydrous)	0.09767
Potassium Chloride	0.4
Sodium Chloride	6.8
Sodium Phosphate Monobasic (anhydrous)	0.122
D-Glucose	1.0
Phenol Red•Na	0.011

Precautions and Disclaimer

REAGENT
For In Vitro Diagnostic Use

Preparation Instructions

Powdered salts are extremely hygroscopic and should be protected from atmospheric 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 solutions. The nature of the supplement may affect storage conditions and shelf life of the solution.

1. Measure out 90% of final required volume of water. Water temperature should be 15-20 °C.
2. While gently stirring the water, add the powdered salts. 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 2.2 g sodium bicarbonate or 29.3 ml of sodium bicarbonate solution [7.5%w/v] for each liter of final volume of salt solution being prepared. Stir until dissolved.
5. While stirring, adjust the pH of the solution 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.
6. Add additional water to bring the solution to final volume.
7. Sterilize immediately by filtration using a membrane with a porosity of 0.22 microns.
8. Aseptically dispense salt solution into sterile container.

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

Procedure
MATERIALS REQUIRED BUT NOT PROVIDED
Water for tissue culture use [W3500]
Sodium Bicarbonate [S5761] or
Sodium Bicarbonate Solution, 7.5% [S8761]
1N Hydrochloric Acid [H9892]
1N Sodium Hydroxide [S2770]
Medium additives as required

Product Profile

Appearance	light to dark pink powder
Moisture content	≤2.0%
Solubility	clear solution at 1x concentration
pH at room temperature [without sodium bicarbonate]	5.2 ± 0.3
pH at room temperature [with sodium bicarbonate]	7.6 ± 0.3
Osmolality [without sodium bicarbonate]	240 mOsm/kg H_2O ± 5%
Osmolality [with sodium bicarbonate]	288 mOsm/kg H_2O ± 5%
Glucose	10.4-12.6%
Endotoxin	≤1.0 EU/ml
Key Element Analysis by ICAP	Analysis has confirmed that key elements are present at concentrations consistent with the formula.

BIOLOGICAL PERFORMANCE CHARACTERISTICS
The ability of this balanced salt solution to maintain the structural integrity of cells in culture was evaluated using an appropriate cell line. There was no evidence of cytotoxicity or uncharacteristic cell morphology. Test results are available upon request.

References
1. Earle, W., (1943) Production of Malignancy In Vitro. IV. The Mouse Fibroblast Cultures and Changes Seen in the Living Cells. J.N.C.I. 4, 165-169.

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