ProductInformation

RPMI-1640 MEDIUM
With L-Glutamine and Without Sodium Bicarbonate
HYBRI-MAX®

Product Number **R5382**
Storage Temperature 2-8°C

Product Description
RPMI-1640 medium was developed by Moore et al., at Roswell Park Memorial Institute, hence the acronym RPMI. The formulation is based on the RPMI-1630 series of media utilizing a bicarbonate buffering system and alterations in the amounts of amino acids and vitamins. RPMI-1640 medium has been used for the culture of normal and neoplastic leukocytes. RPMI-1640 when properly supplemented, has demonstrated wide applicability for supporting growth of many types of cell cultures, including fresh human lymphocytes in the 72-hour phytohemagglutinin (PHA) stimulation assay.

RPMI-1640, Product No. R5382 is one of the cell culture media available from Sigma. The selection of a nutrient medium 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
L-Arginine [Free Base]	0.2
L-Asparagine [Anhydrous]	0.05
L-Aspartic Acid	0.02
L-Cystine•2HCl	0.0652
L-Glutamic Acid	0.02
L-Glutamine	0.3
Glycine	0.01
L-Histidine [Free Base]	0.015
Hydroxy-L-Proline	0.02
L-Isoleucine	0.05
L-Leucine	0.05
L-Lysine•HCl	0.04
L-Methionine	0.015
L-Phenylalanine	0.015
L-Proline	0.02
L-Serine	0.03
L-Threonine	0.02
L-Tryptophan	0.005
L-Tyrosine•2Na•2H_2O	0.02883
L-Valine	0.02
Biotin	0.0002
Choline Chloride	0.003
Folic Acid	0.001
myo-Inositol	0.035
Niacinamide	0.001
D-Pantothenic Acid Hemicalcium	0.00025
PABA	0.001
Pyridoxine•HCl	0.001
Riboflavin	0.0002
Thiamine•HCl	0.001
Vitamin B12	0.000005
Calcium Nitrate•4 H_2O	0.1
Magnesium Sulfate [Anhydrous]	0.04884
Potassium Chloride	0.4
Sodium Chloride	6.0
Sodium Phosphate Dibasic [Anhydrous]	0.8
D-Glucose	2.0
Glutathione, Reduced	0.001
Phenol Red•Na	0.0053

Precautions and Disclaimer
REAGENT
For In Vitro Diagnostic Use

Preparation Instructions
Powdered media 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 solution of medium is not recommended as precipitates may form.

Supplements can be added prior to filtration or introduced aseptically to sterile medium. The nature of the supplement may affect storage conditions and shelf life of the medium.

1. Measure out 90% of final required volume of water. Water temperature should be 15-20EC.
2. 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.

NOTE: It may be necessary to lower the pH to 4.0 with 1N HCl to completely dissolve this product. After it has dissolved completely, the pH can be raised to 7.2 with 1N NaOH prior to the addition of sodium bicarbonate.

4. To the solution in step 3, add 2.0 g sodium bicarbonate or 26.7 ml of sodium bicarbonate solution [7.5%w/v] for each liter of final volume of medium being prepared. Stir until dissolved.
5. 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.
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 medium into sterile container.

Storage/Stability

Store the dry powdered medium 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 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 medium. Product label bears expiration date.

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	off-white powder
Moisture content	≤ 2.0%
Solubility	clear solution at 1x concentration after adjustment to pH 4.0 with 1N HCl
pH at room temperature* [without sodium bicarbonate]	8.1 ± 0.3
pH at room temperature* [with sodium bicarbonate]	8.1 ± 0.3
Osmolality* [without sodium bicarbonate]	237 mOsm/kg H2O ± 5%
Osmolality* [with sodium bicarbonate]	279 mOsm/kg H2O ± 5%
Endotoxin	≤ 0.5 EU/ml at 1x
Amino Acid Analysis by HPLC	Analysis has confirmed that that amino acids are present at concentrations consistent with the formula.
Key Element Analysis by ICAP	Analysis has confirmed that key elements are present at concentrations consistent with the formula.

BIOLOGICAL PERFORMANCE CHARACTERISTICS

Biological performance is assessed using an appropriate cell line(s). Growth studies are carried through 2 subculture generations. Cells are counted and growth is plotted as a logarithmic function of time in culture. Seeding efficiencies, doubling time, and final cell densities are determined. During the testing period cultures are examined microscopically for atypical morphology and evidence of cytotoxicity. This product is also assessed for its ability to support clonal growth and maintenance of hybridoma cells. Test results are available upon request.

*pH and osmolality determined without adjustment to pH 4.0 with 1N HCl.

References

1. Moore, G.E., Gerner, R.E. and Franklin, H.A., (1967). Culture of Normal Human Leukocytes. JAMA. 199, 519-524.
2. Moore, G.E. and Woods L.K., (1976). Culture Media for Human Cells- RPMI 1603, RPMI 1634, RPMI 1640 and GEM 1717. Tissue Culture Association Manual. 3, 503-508.
3. Moore, G.E. Gerner, R.E. and Minowada, J., (1967). Studies of Normal and Neoplastic Cells. Studies of Normal and Neoplastic Human Hematopoietic Cells In Vitro. Twenty-first Annual Symposium on Fundamental Cancer Research. February, 41-63.
4. Moore, G.E. and Kitamura, H., (1968). Cell Line Derived from Patient with Myeloma. NY State Journal of Medicine. 68, 2054-2060.

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