



DULBECCO'S MODIFIED EAGLE'S MEDIUM NUTRIENT MIXTURE F-12 HAM

With L-Glutamine and 15 mM HEPES, Without Sodium Bicarbonate

Product Number **D8900**

Product Description

A variety of cell lines have been cultured in serum-free medium that contained, instead of serum, a supplement of nutrients, growth factors and hormones. Although the supplements and their concentrations are specific for the type of cell under study, the medium found to be most satisfactory for studies of this type was a 1:1 mixture of Dulbecco's Modified Eagle's Medium (DME) and Ham's F-12 Nutrient Mixture. HEPES buffer is included in the formulation at a final concentration of 15 mM to compensate for the loss of buffering capacity incurred by eliminating serum.

Components	g/L
Calcium Chloride anhydrous	0.1166
Cupric Sulfate•5H ₂ O	0.0000013
Ferric Nitrate•9H ₂ O	0.00005
Ferrous Sulfate•7H ₂ O	0.000417
Magnesium Chloride (anhydrous)	0.028661184
Magnesium Sulfate (anhydrous)	0.04884
Potassium Chloride	0.3118
Sodium Chloride	6.996
Sodium Phosphate Dibasic (anhydrous)	0.07102
Sodium Phosphate Monobasic (anhydrous)	0.0543
Zinc Sulfate•7H ₂ O	0.000432
L-Alanine	0.00445
L-Arginine•HCl	0.1475
L-Asparagine•H ₂ O	0.0075
L-Aspartic Acid	0.00665
L-Cysteine•HCl•H ₂ O	0.01756
L-Cystine•2HCl	0.03129
L-Glutamic Acid	0.00735
L-Glutamine	0.365
Glycine	0.01875
L-Histidine•HCl•H ₂ O	0.03148
L-Isoleucine	0.05447
L-Leucine	0.05905
L-Lysine•HCl	0.09125
L-Methionine	0.01724
L-Phenylalanine	0.03548
L-Proline	0.01725
L-Serine	0.02625
L-Threonine	0.05345
L-Tryptophan	0.00902
L-Tyrosine•2Na•2H ₂ O	0.05579
L-Valine	0.05285
D-Biotin	0.0000035
Choline Chloride	0.00898
Folic Acid	0.00266
myo-Inositol	0.0126
Niacinamide	0.00202
D-Pantothenic Acid (hemicalcium)	0.00224
Pyridoxal•HCl	0.002

Pyridoxine•HCl	0.000031
Riboflavin	0.000219
Thiamine•HCl	0.00217
Vitamin B-12	0.00068
D-Glucose	3.15
HEPES	3.5745
Hypoxanthine	0.0021
Linoleic Acid	0.000042
Phenol Red•Na	0.00863
Putrescine•2HCl	0.000081
Pyruvic Acid•Na	0.055
Thioctic Acid	0.000105
Thymidine	0.000365

Precautions and Disclaimer

REAGENT

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

Preparation Instructions

Powdered media are hygroscopic and should be protected from moisture. The entire contents of each package should be used 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.

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 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 1.2 g sodium bicarbonate or 16 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 and 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 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]

1N Hydrochloric Acid [H9892]

1N Sodium Hydroxide [S2770]

Medium additives as required

Reference

1. Barnes, D. and Sato, G. (1980). Methods for Growth of Cultured Cells in Serum-Free Medium. Analytical Biochemistry. 102, 255-270.

Revised: April 2007

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