Nutrient Mixture F-12 COON'S MODIFICATION
With L-Glutamine and 0.863 mg/L Zinc Sulfate, without $NaHCO_3$
Product Number **F6636**

Product Description
Coon's Modification of Ham's F-12 was developed for culturing hybrid cells that were produced by viral fusion. The modification consists of doubling the amino acids and pyruvate, and including ascorbic acid. The salt concentrations have been altered as well. This formula contains 0.863 mg zinc sulfate per liter, which may render it unsuitable for culturing mouse L-cells.

Components	g/L
Calcium Chloride (anhydrous)	0.1245
Cupric Sulfate•$5H_2O$	0.0000025
Ferrous Sulfate•$7H^2O$	0.000834
Magnesium Chloride (anhydrous)	0.046659
Magnesium Sulfate (anhydrous)	0.02528
Potassium Chloride	0.305
Potassium Phosphate Monobasic	0.06124
Sodium Chloride	7.517
Sodium Phosphate Dibasic (anhydrous)	0.1324
Zinc Sulfate•$7H_2O$	0.000863
L-Alanine	0.018
L-Arginine•HCl	0.422
L-Asparagine•H_2O	0.03
L-Aspartic Acid	0.026
L-Cysteine•HCl•H_2O	0.07026
L-Glutamic Acid	0.03
L-Glutamine	0.292
Glycine	0.016
L-Histidine•HCl•H_2O	0.042
L-Isoleucine	0.0078
L-Leucine	0.0262
L-Lysine•HCl	0.073
L-Methionine	0.009
L-Phenylalanine	0.01
L-Proline	0.07
L-Serine	0.021
L-Threonine	0.0238
L-Tryptophan	0.004
L-Tyrosine•2Na•$2H_2O$	0.01586
L-Valine	0.0234
L-Ascorbic Acid•Na	0.015
D-Biotin	0.0000073
Choline Chloride	0.01396
Folic Acid	0.00132
myo-Inositol	0.01802
Niacinamide	0.00004
D-Pantothenic Acid (hemicalcium)	0.000238
Pyridoxine•HCl	0.00006
Riboflavin	0.00004
Thiamine•HCl	0.000337
Vitamin B-12	0.00136
D-Glucose	1.802
Hypoxanthine	0.00404
Linoleic Acid	0.00009
Phenol Red•Na	0.00125
Putrescine•2HCl	0.000161
Pyruvic Acid•Na	0.22
DL-6,8-Thioctic Acid	0.000206
Thymidine	0.0007

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 2.68 g sodium bicarbonate or 35.7 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 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. Coon, H.G. and Weiss, M.C. (1969). A Quantitative Comparison of Formation of Spontaneous and Virus-Produced Viable Hybrids. PNAS, 62, 852-859.

Revised: April 2007

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