ProductInformation

SERUM-FREE AND PROTEIN-FREE HYBRIDOMA MEDIUM
With L-Glutamine and MOPS Buffer Without Sodium Bicarbonate

Product Number **S2772**
Storage Temperature 2-8°C

Product Description
In 1975 Kohler and Milstein were the first to report an immortal hybridoma cell line capable of monoclonal antibody production. Since then defined media capable of supporting the growth of hybridoma and myeloma cells in the absence of serum or protein have been developed. Sigma's Serum-free and Protein-free Hybridoma medium (S 2772) is based on a modification of Ham's Nutrient Mixture F-12 which is further modified to contain additional components (see formula) and a MOPS buffering system. This medium is designed to support the growth of hybridomas, but may not support the growth of myelomas which require cholesterol. It is ideal for applications where exogenous proteins are undesirable, such as in the production and purification of monoclonal antibodies or other cellular proteins.

SERUM-FREE AND PROTEIN-FREE HYBRIDOMA MEDIUM, Product No. S 2772 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

Aluminum Chloride•6H_2O	0.000001
Ammonium Metavanadate	0.0000006
Barium Chloride	0.000002
Calcium Chloride•2H_2O	0.0441
Cobalt Chloride•6H_2O	0.000002
Chromic Potassium Sulfate	0.000001
Cupric Sulfate•5H_2O	0.0000051
Ferrous Sulfate•7H_2O	0.000834
Germanium Dioxide	0.0000005
Lithium Chloride	0.01
Magnesium Chloride [Anhydrous]	0.0576
Manganese Chloride [Anhydrous]	0.0000001
Molybdic Acid•2Na•2H_2O	0.0000001
Nickel Nitrate•6H_2O	0.0000002
Potassium Bromide	0.0000001
Potassium Chloride	0.224
Potassium Iodide	0.0000001
Rubidium Chloride	0.00000001
Silver Chloride	0.0000000044
Sodium Chloride	7.599
Sodium Fluoride	0.000004
Sodium Phosphate Dibasic[Anhydrous]	0.39739
Sodium Selenite	0.00003
Stannous Chloride	0.0000001
Titanium Chloride	0.000001
Zinc Sulfate•7H_2O	0.000863
L-Alanine	0.009
L-Arginine•HCl	0.211
L-Asparagine•H_2O	0.03401
L-Aspartic Acid	0.0133
L-Citrulline	0.005
L-Cysteine•HCl•H_2O	0.035
L-Glutamic Acid	0.0147
L-Glutamine	0.396
Glycine	0.00751
L-Histidine•HCl•H_2O	0.071
L-Isoleucine	0.164
L-Leucine	0.133
L-Lysine•HCl	0.109
L-Methionine	0.015
L-Ornithine	0.008
L-Phenylalanine	0.055
L-Proline	0.0345
L-Serine	0.051
L-Threonine	0.0119
L-Tryptophan	0.005
L-Tyrosine•2Na•2H_2O	0.0312
L-Valine	0.091
D-Biotin	0.00011
Choline Chloride	0.01396
Flavin Adenine Dinucleotide	0.00002
Folic Acid	0.00132
myo-Inositol	0.018
Niacinamide	0.0065
D-Pantothenic Acid [Hemicalcium]	0.0035
Pyridoxine•HCl	0.0002
Riboflavin	0.000038
Thiamine•HCl	0.00034
α-Tocopherol Acetate	0.00015
Vitamin B12	0.00136
Adenine	0.0004
Citric Acid•H_2O	0.025
Dilinoleoyl Phosphatidylcholine	0.0005
Distearyol Phosphatidylcholine	0.0005
Ethanolamine•3HCl	0.003
EDTA•2Na	0.0055
Oxalacetic Acid	0.005
Progesterone	0.000006
Sodium Nitroprusside	0.0057
Spermine•3HCl	0.0005
Taurine	0.03
Tween 80	0.0002
Glucose	5.202
Hypoxanthine	0.00408
Linoleic Acid	0.000084
Thioctic Acid	0.000121
Phenol Red•Na	0.0042
Putrescine•HCl	0.000161

Pyruvic Acid•Na	0.11
Thymidine	0.0008
MOPS	3.135

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 15-20°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 2.25 g sodium bicarbonate or 30.0 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.

Procedure
Water for tissue culture use [W 3500]
Sodium Bicarbonate [S 5761] or
Sodium Bicarbonate Solution, 7.5% [S 8761]
1N Hydrochloric Acid [H 9892]
1N Sodium Hydroxide [S 2770]
Medium additives as required

Product Profile

Appearance	off-white powder
Moisture content	≤2.0%
Solubility	clear solution at 1x concentration
pH at room temperature [without sodium bicarbonate]	5.8 ± 0.3
pH at room temperature [with sodium bicarbonate]	6.8 ± 0.3
Osmolality [without sodium bicarbonate]	309 mOsm/kg H_2O ± 5%
Osmolality [with sodium bicarbonate]	348 mOsm/kg $H_2$2O ± 5%
Amino Acid Analysis by HPLC	Analysis has confirmed 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.
Endotoxin	≤0.5 EU/ml

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. Test results are available upon request.

References

1. Ham, R.G. (1965). Clonal growth of mammalian cells in a chemically defined synthetic medium. Proc. Natl. Acad. Sci. USA. 53,288-293.
2. Myoken, Y., Okamoto, T., Osaki, T., Yabumoto, M., Sato, G.H., Takada, K. and Sato, J.D. (1989). An alternative method for the isolation of NS-1 hybridomas using cholesterol auxotrophy of NS-1 myeloma cells. In Vitro. 25,477-480.