ProductInformation

H-Y MEDIUM
With L-Glutamine, Bovine Insulin, Oxaloacetate, and Sodium Pyruvate
Without Sodium Bicarbonate
HYBRI-MAX®

Product Number **H9014**
Storage Temperature 2-8°C

Product Description
H-Y Medium is a rich, complex medium specifically designed to support hybrid cells in culture. It is a mixture containing seven parts of Dulbecco's Modified Eagle's Medium with 4500 mg glucose/L and one part NCTC 109. H-Y Medium also contains oxaloacetate, sodium pyruvate, and bovine insulin.

H-Y MEDIUM [DME/NCTC 109 7:1 Mixture], Product No. H9014 HYBRI-MAX® 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-α -Amino-n-Butyric Acid	0.00068875
L-Alanine	0.003935
L-Arginine•HCl	0.077395
L-Asparagine H_2O	0.00114875
L-Aspartic Acid	0.00123875
L-Cystine•2HCl	0.056485
L-Cysteine•HCl•H_2O	0.03621375
D-(+)-Glucosamine•HCl	0.00048125
L-Glutamic Acid	0.0010325
L-Glutamine	0.52796625
Glycine	0.02793875
L-Histidine HCl•H_2O	0.04008125
Hydroxy-L-Proline	0.00051125
L-Isoleucine	0.09413
L-Leucine	0.09443
L-Lysine•HCl	0.13255375
L-Methionine	0.026805
L-Ornithine•HCl	0.00117625
L-Phenylalanine	0.05981625
L-Proline	0.00076625
L-Serine	0.03809375
Taurine	0.0005225
L-Threonine	0.08549125
L-Tryptophan	0.161875
L-Tyrosine 2Na•2H_2O	0.09377875
L-Valine	0.085375
L-Ascorbic Acid•Na	0.00625
D-Biotin	0.000003125
Choline Chloride	0.00365625
Coenzyme A	0.0003125
Cocarboxylase	0.000125
2'Deoxyadenosine	0.00125
2'Deoxyguanosine	0.00125
2'Deoxycytidine•HCl	0.00125
Ergocalciferol	0.00003125
FAD 2Na	0.000125
Folic Acid	0.003503125
D-Glucuronic Acid Lactone	0.000225
D-Glucuronic Acid•Na	0.000225
Glutathione•2Na[oxidized]	0.0025
myo-Inositol	0.006315625
Bovine Insulin	0.00833
Menadione•Na Bisulfite	0.000005
5'Methylcytosine•HCl	0.0000125
Niacinamide	0.003507812
β -NADP•2Na	0.000125
β -NAD•Na	0.000875
Nicotinic Acid	0.000007812
Oxalacetic Acid	0.15
PABA	0.000015625
D-Pantothenic Acid•Ca	0.003503125
Pyridoxal•HCl	0.003507812
Pyridoxine•HCl	0.000007812
Retinol Acetate	0.00003125
Riboflavin	0.000353125
DL-α -Tocopherol Phosphate•Na	0.000003125
Thiamine•HCl	0.00353125
Thymidine	0.00125
Tween 80	0.0015625
UTP•Na	0.000125
Vitamin B-12	0.00125
Calcium Chloride•2H_2O	0.265
Ferric Nitrate•9H_2O	0.0000875
D-(+)-Glucose	4.0625
Magnesium Sulfate [Anhydrous]	0.1
Potassium Chloride	0.4
Sodium Acetate [Anhydrous]	0.00375
Sodium Chloride	6.45
Sodium Phosphate Monobasic [Anhydrous]	0.111
Sodium Pyruvate	0.05
Phenol Red•Na	0.0164125

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 3.5 g sodium bicarbonate or 46.6 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
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
pH at room temperature [without sodium bicarbonate]	3.4 ± 0.3
pH at room temperature [with sodium bicarbonate]	7.2 ± 0.3
Osmolality [without sodium bicarbonate]	260 mOsm/kg H_2O ± 5%
Osmolality [with sodium bicarbonate]	320 mOsm/kg H_2O ± 5%
Endotoxin	≤0.5 EU/ml at 1x
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.

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.

References

1. Kennett, R.H., McKearn, T.J., and Bechtol, K.B.(1980). Monoclonal Antibodies: Hybridomas, A New Dimension in Biological Analyses. 365-371.
2. Kennett, R.H.(1978) Hybridoma Plasmacytoma Production: Fusions with Adult Spleen Cells, Monoclonal Spleen Fragments, Neonatal Spleen Cells and Human Spleen Cells. 81, 77.

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