



Product Information

GRACE'S INSECT MEDIUM With L-Glutamine, Without Sodium Bicarbonate

Product No. **G 9771**
Store at 2-8°C

Product Description

Grace's medium was originally formulated to support the growth of cells derived from the Australian emperor gum moth, *Antherea eucalypti*. The medium is a modification of Wyatt's medium to more closely resemble *Antherea* hemolymph. The cells established by Grace using this medium were the first continuous lines developed. The basal medium, when properly supplemented, has been used to culture cells derived from a variety of insects including several species of lepidopterans as well as some dipterans. The medium is primarily used as a basal medium for the growth and maintenance of cell lines derived from lepidopterans.

GRACE'S INSECT MEDIUM, Product No. G9771 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
Calcium Chloride (anhydrous)	1.0
Magnesium Chloride (anhydrous)	1.068189
Magnesium Sulfate (anhydrous)	1.357858
Potassium Chloride	2.24
Sodium Phosphate Monobasic	0.876923
β-Alanine	0.2
L-Alanine	0.225
L-Arginine•HCl	0.7
L-Aspartic Acid	0.35
L-Asparagine	0.35
L-Cystine•2HCl	0.025
L-Glutamic Acid	0.6
L-Glutamine	0.6
Glycine	0.65
L-Histidine	2.5
L-Isoleucine	0.05
L-Leucine	0.075
L-Lysine•HCl	0.625
L-Methionine	0.05
L-Phenylalanine	0.15

L-Proline	0.35
L-Serine	0.55
L-Threonine	0.175
L-Tryptophan	0.1
L-Tyrosine•2Na	0.07202
L-Valine	0.1
p-Aminobenzoic Acid	0.00002
D-Biotin	0.00001
Choline Chloride	0.0002
Folic Acid	0.00002
myo-Inositol	0.00002
Niacin	0.00002
D-Pantothenic Acid (hemicalcium)	0.00002
Pyridoxine•HCl	0.00002
Riboflavin	0.00002
Thiamine•HCl	0.00002
D(-)-Fructose	0.4
Fumaric Acid, free acid	0.055
D(+)-Glucose	0.7
α-Ketoglutaric Acid	0.37
L(-)-Malic Acid, free acid	0.67
Succinic Acid	0.06
Sucrose	26.68

Precautions and Disclaimer

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

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 0.35 g sodium bicarbonate or 4.7 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. The medium will have an osmotic pressure of 315 mOsm \pm 5%. The optimal osmotic pressure is species specific and has been found to be at or near the osmotic pressure of the hemolymph of the insect from which the cells were derived. While many cell lines exhibit a tolerance for a broad range of osmotic pressures some cell lines require a narrow range for growth. The optimal osmotic pressure for lepidopteran species is generally in the range of 300-380 mOsm with values of 340-360 mOsm frequently cited for culture of lepidopteran cells. The osmotic pressure of the medium can be increased 10 mOsm by addition of potassium chloride [0.40 g of salt or 2 ml of a 20% [w/v] solution] OR sodium chloride [0.30 g of salt or 2 ml of a 15 % [w/v] solution] for EACH liter of final volume of medium being prepared. Stir until dissolved. The osmotic pressure of the medium can be decreased 10 mOsm by the addition of 27.8 ml of water for EACH liter of final volume of medium prepared.
8. Sterilize immediately by filtration using a membrane with a porosity of 0.22 microns.
9. Aseptically dispense medium into sterile container.

Product Storage

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]

Potassium Chloride [P5405] or
 Potassium Chloride Solution, 20% [P1302]
 Sodium Chloride [S5886] or
 Sodium Chloride Solution, 15% [S1772]
 Medium additives as required

Product Profile

Appearance	off-white powder
Moisture content	\leq 2.0%
Solubility	clear solution at 1x concentration
pH at RT	4.5 \pm 0.3 [without sodium bicarbonate]
pH at RT	4.9 \pm 0.3 [with sodium bicarbonate]
Osmolality	300 mOsm/kg H ₂ O \pm 5% [without sodium bicarbonate]
Osmolality	315 mOsm/kg H ₂ O \pm 5% [with sodium bicarbonate]
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. Test results are available upon request.

References

1. Grace, T.D.C. (1962). Establishment of four strains of cells from insect tissues grown *in vitro*. Nature 195:788-789.
2. Grace, T.D.C. (1967). Establishment of a line of cells from the silkworm, *Bombyx mori*. Nature 216, 613.
3. Grace, T.D.C. (1966). Establishment of a line of mosquito, (*Aedes aegypti*) cells grown *in vitro*. Nature. 211, 366-367.

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