

# Product Information

## Grace's Insect Medium

Original Formula, with 4.1 mM L-glutamine, without calcium chloride, without sodium bicarbonate

CATALOG NO. 56902C

### Description

Grace's Insect Medium was developed in 1962 and has been shown, with the addition of serum, to support the growth of insect cells, including *Spodoptera frugiperda*.

### Precautions

Use aseptic technique when handling or supplementing this medium after filtration. This product is for further manufacturing use. THIS PRODUCT IS NOT INTENDED FOR HUMAN OR THERAPEUTIC USE.

### Storage

Store dry powder medium at 2 to 8 C. Do not use after the expiration date. Store hydrated medium protected from light at 2 to 8 C.

### Indications of Deterioration

Dry powder medium should be free flowing. Do not use if powder is caked. Prepared medium should be clear of particulates and flocculent material. Do not use if liquid medium is cloudy or contains precipitate. Other evidence of deterioration may include color change or degradation of physical or performance characteristics.

### Formulation

Component (all components measured in mg/L)	
<b>INORGANIC SALTS</b>	
Magnesium chloride anhydrous	1067.860
Magnesium sulfate anhydrous	1357.670
Potassium chloride	2240.000
Sodium phosphate monobasic monohydrate	1013.000
<b>VITAMINS</b>	
Biotin	0.010
D-calcium pantothenate	0.020
Choline chloride	0.200
Folic acid	0.020
i-inositol	0.020
Niacin	0.020
PABA	0.020
Pyridoxine HCl	0.020
Riboflavin	0.020
Thiamine HCl	0.020
<b>AMINO ACIDS</b>	
β-alanine	200.000
L-alanine	225.000
L-arginine HCl	700.000
L-asparagine monohydrate	397.730
L-aspartic acid	350.000
L-cystine 2HCl	28.680
L-glutamic acid	600.000
L-glutamine	600.000
Glycine	650.000
L-histidine free base	2500.000
L-isoleucine	50.000
L-leucine	75.000
L-lysine HCl	625.000
L-methionine	50.000
L-phenylalanine	150.000
L-proline	350.000
DL-serine	1100.000
L-threonine	175.000
L-tryptophan	100.000
L-tyrosine 2Na dihydrate	72.630
L-valine	100.000
<b>OTHER</b>	
Dextrose anhydrous	700.000
D-fructose	400.000
Fumaric acid	55.000
α-Ketoglutaric acid	370.000
L-malic acid	670.000
Succinic acid free acid	60.000
D+Sucrose	26680.000
ADD: Calcium chloride anhydrous	1000.000
ADD: Sodium bicarbonate	350.000
Grams of powder per liter	43.713

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## Preparation Instructions

1. Measure 80 - 90% of the final volume of cell culture grade water (Catalog No. 59900C) into an appropriate size mixing vessel. Water temperature should be 15 to 30 C. Do not heat water.
2. Add the dry powder medium to the water. Rinse the original package with a small amount of cell culture grade water to remove all traces of powder and add to the solution. Mix until completely dissolved.
3. For each liter being prepared, add 350.00 mg/L of sodium bicarbonate (Catalog No. 90421C) or 4.7 mL/L of sodium bicarbonate solution 7.5% (Catalog No. 59221C). Mix until completely dissolved.
4. For calcium supplementation, prepare a 20% calcium chloride stock solution by dissolving 10.0 g of calcium chloride anhydrous (CaCl<sub>2</sub>) in 40.0 mL of cell culture grade water.

**NOTE:** The reaction is exothermic.

QS the solution to a final volume of 50.0 mL. For each liter of medium being prepared, add 5.0 mL of the stock solution to achieve the required amount (1.0 g/L) of calcium chloride. SAFC Biosciences recommends adding the pre-dissolved calcium chloride to the medium prior to adjusting the pH as directed in step 5 below.

5. While stirring the solution in Step 3, adjust the pH to 6.2 using KOH 1N. Use of NaOH may cause precipitation.
6. Add cell culture grade water to the solution in Step 4 to bring it to the final volume. Keep the vessel closed until the solution is filtered to avoid fluctuations in pH.
7. Sterilize the solution using a membrane filter with a pore size of 0.22 µm or less. A peristaltic pump or an inert gas such as nitrogen can be used to provide positive pressure at 3 - 15 psi. Do not use CO<sub>2</sub> gas.
8. Sterile solutions should be dispensed aseptically into sterile containers. Store protected from light at 2 to 8 C.
9. Supplements, such as antibiotics, can be added to the sterilized solution using aseptic technique. Storage conditions and shelf life of the supplemented product may be affected by the nature of the supplements. Sterile serum should not be refiltered before or after being added to sterile medium because growth promoting capacity may be reduced upon refiltration.

**NOTE:** Dry powdered medium is extremely hygroscopic and must be protected from atmospheric moisture. We recommend that the entire contents of each package be used immediately after opening.

## Characteristics

### Appearance

Off-white free-flowing powder

### Bioburden

≤ 100 CFU/100 mL

### Endotoxin

≤ 1.0 EU/mL

### Osmolality (as supplied)

245 - 285 mOsm/kg H<sub>2</sub>O

### Osmolality (with NaHCO<sub>3</sub>)

260 - 300 mOsm/kg H<sub>2</sub>O

### pH (as supplied)

4.3 - 4.7

### pH (with NaHCO<sub>3</sub>)

4.7 - 5.1

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