

Product Information

Iscove's Modified Dulbecco's Medium

with 4.0 mM L-glutamine, with 25 mM HEPES, without sodium bicarbonate

CATALOG NO. 56479C

Description

In 1976, Guilbert and Iscove published the formula for their modification of Dulbecco's Modified Eagle's Medium, creating Iscove's Modified Dulbecco's Medium (IMDM). It replaced ferric nitrate with potassium nitrate, and included selenium, additional amino acids and vitamins, sodium pyruvate and HEPES buffer. This medium, when supplemented with albumin, lecithin and transferrin, supported the growth of precursor cells of erythrocytes and macrophages in the absence of serum. Since then, IMDM has been used to culture a wide variety of cells including lymphocytes and hybrid cells for antibody production.

This product contains 25 mM HEPES to provide additional buffering capacity to the medium. HEPES, a zwitterionic buffer, has a pKa of 7.3 at 37 C, which is more compatible with most culture systems than the pKa of NaHCO₃ usually 6.2 under similar conditions. HEPES should not be used in place of bicarbonate, however, as most cells require CO₂, which is normally supplied in the form of the bicarbonate ion. HEPES will reduce sudden, drastic pH shifts, but as with other buffers, it cannot prevent pH shifts altogether.

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.

Formulation

Component (all components measured in mg/L)	
INORGANIC SALTS	
Calcium chloride anhydrous	165.000
Magnesium sulfate anhydrous	97.670
Potassium chloride	330.000
Potassium nitrate	0.076
Sodium chloride	4505.000
Sodium phosphate monobasic monohydrate	125.000
Sodium selenite	0.0173
VITAMINS	
Biotin	0.013
D-calcium pantothenate	4.000
Choline chloride	4.000
Cyanocobalamin	0.013
Folic acid	4.000
i-inositol	7.200
Niacinamide	4.000
Pyridoxal HCl	4.000
Riboflavin	0.400
Thiamine HCl	4.000
AMINO ACIDS	
L-alanine	25.000
L-arginine HCl	84.000
L-asparagine monohydrate	28.400
L-aspartic acid	30.000
L-cystine 2HCl	91.240
L-glutamic acid	75.000
L-glutamine	584.000
Glycine	30.000
L-histidine HCl monohydrate	42.000
L-isoleucine	105.000
L-leucine	105.000
L-lysine HCl	146.000
L-methionine	30.000
L-phenylalanine	66.000
L-proline	40.000
L-serine	42.000
L-threonine	95.000
L-tryptophan	16.000
L-tyrosine 2Na dihydrate	103.790
L-valine	94.000
OTHER	
Dextrose anhydrous	4500.000
HEPES	5958.000
Phenol red sodium salt	15.930
Sodium pyruvate	110.000
ADD: Sodium bicarbonate	3024.000
Grams of powder per liter	17.671

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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.

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 3.024 g/L of sodium bicarbonate (Catalog No. 90421C) or 40.3 mL/L of sodium bicarbonate solution 7.5% (Catalog No. 59221C). Mix until completely dissolved.
4. While stirring the solution in Step 3, adjust the pH to 6.7 - 6.9 using NaOH 1N (Catalog No. 59223C) or HCl 1N. The pH of bicarbonate buffered solutions usually rises 0.1 - 0.2 units during filtration.
5. 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.
6. 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 CO₂ gas.
7. Sterile solutions should be dispensed aseptically into sterile containers. Store protected from light at 2 to 8 C.
8. 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 powder 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)

Refer to Certificate of Analysis for lot-specific value

pH (as supplied)

4.6 - 5.0

References

1. Guilbert, L. and Iscove, N., *Nature* (1976) 36:405.
2. Iscove, N. and Melchers, F., *J Exp Med* (1978) 199:519.

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Issued December 2006 P56479
1203 1005 0406 0606 0906

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