

# Product Information

## Dulbecco's Modified Eagle's Medium/ Ham's Nutrient Mixture F12

with 3151 mg/L dextrose, with 2.5 mM L-glutamine, with 15 mM HEPES, with 55 mg/L sodium pyruvate,  
without sodium bicarbonate

CATALOG NO. 56495C

### Description

Studies to determine the nutritional requirements of many cells have been in progress since Eagle's first reports. The major essential nutrients were identified and work became focused on the media requirements of individual cell types. Many media designed for these purposes are now available. Among the first of these media, developed initially to study hormonal requirements of cells in culture, was a mixture of Dulbecco's Modified Eagle's Medium and Ham's Nutrient Mixture F12, known as DMEM/F12. This product is at a 1:1 ratio of the two media.

This product contains 15 mM HEPES to provide additional buffering capacity to the medium. A zwitterionic buffer, HEPES has a pKa of 7.3 at 37 C, which is more compatible with most culture systems than the pKa of sodium bicarbonate which is 6.2 under similar conditions. HEPES will reduce sudden, drastic pH shifts, but as with other buffers, it will not prevent pH shifts entirely.

### Formulation

Component (all components measured in mg/L)	
<b>INORGANIC SALTS</b>	
Calcium chloride anhydrous	116.610
Cupric sulfate pentahydrate	0.00125
Ferric nitrate nonahydrate	0.050
Ferrous sulfate heptahydrate	0.417
Magnesium chloride anhydrous	28.610
Magnesium sulfate anhydrous	48.840
Potassium chloride	311.800
Sodium chloride	6999.500
Sodium phosphate dibasic anhydrous	71.020
Sodium phosphate monobasic monohydrate	62.500
Zinc sulfate heptahydrate	0.4315

Component continued (all components measured in mg/L)	
<b>VITAMINS</b>	
Biotin	0.00365
D-calcium pantothenate	2.240
Choline chloride	8.980
Cyanocobalamin	0.680
Folic acid	2.650
i-inositol	12.600
Niacinamide	2.0185
Pyridoxal HCl	2.000
Pyridoxine HCl	0.031
Riboflavin	0.219
Thiamine HCl	2.170
<b>AMINO ACIDS</b>	
L-alanine	4.455
L-arginine HCl	147.500
L-asparagine monohydrate	7.500
L-aspartic acid	6.650
L-cysteine HCl monohydrate	17.560
L-cystine 2HCl	31.290
L-glutamic acid	7.350
L-glutamine	365.000
Glycine	18.750
L-histidine HCl monohydrate	31.480
L-isoleucine	54.470
L-leucine	59.050
L-lysine HCl	91.250
L-methionine	17.240
L-phenylalanine	35.480
L-proline	17.250
L-serine	26.250
L-threonine	53.450
L-tryptophan	9.020
L-tyrosine 2Na dihydrate	55.790
L-valine	52.850
<b>OTHER</b>	
Dextrose anhydrous	3151.000
HEPES	3575.000
Hypoxanthine sodium salt	2.390
Linoleic acid	0.042
DL- $\alpha$ -Lipoic acid	0.105
Phenol red sodium salt	8.602
Putrescine 2HCl	0.081
Sodium pyruvate	55.000
Thymidine	0.365
ADD: Sodium bicarbonate	1200.000
Grams of powder per liter:	15.576

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## 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 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.

## Preparation Instructions

1. Measure 80 - 90% of the final volume of cell culture grade water 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 slowly. 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 of DMEM/F12 (Modified) being prepared, add 1.20 g/L of sodium bicarbonate (Catalog No. 90421C) or 16.0 mL of sodium bicarbonate solution 7.5% (Catalog No. 59221C) solution. Mix until completely dissolved.
4. While stirring the solution in Step 3, adjust the pH to 6.9 - 7.1 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 0.2  $\mu\text{m}$  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.

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

$\leq 100$  CFU/100 mL

### Endotoxin

$\leq 1.0$  EU/mL

### Osmolality (as supplied)

255 - 295 mOsm/kg H<sub>2</sub>O

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

285 - 325 mOsm/kg H<sub>2</sub>O

### pH (as supplied)

5.8 - 6.2

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

6.8 - 7.2

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