Iscove’s Modified Dulbecco’s Medium (IMDM)

With 25mM HEPES buffer
Without L-Glutamine and Sodium bicarbonate

Product Code: AT070A

Product Description:
Iscove’s Modified Dulbecco’s Medium is an enriched modification of Dulbecco’s Modified Eagle’s Medium wherein serum can be partially or totally replaced by chemically defined substances. The medium contains additional amino acids, sodium selenite, sodium pyruvate, vitamins and inorganic salts. Potassium nitrate is substituted by ferric nitrate. IMDM was the first medium utilizing HEPES buffer. The medium when appropriately supplemented supports good growth of precursor cells of erythrocytes and macrophages. The medium also supports good growth of T and B-lymphocytes and a variety of hybrid cells under serum free or reduced serum conditions.

AT070A is Iscove’s Modified Dulbecco’s Medium with 25mM HEPES. HEPES, a zwitterionic buffer having a pKa of 7.3 at 37°C prevents the initial rise in pH that tends to occur at the initiation of a culture and increases the buffering capacity of the medium. It does not contain L-glutamine. Users are advised to review the literature for recommendations regarding medium supplementation and physiological growth requirements specific for different cell lines.

Composition:

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INORGANIC SALTS</strong></td>
<td></td>
</tr>
<tr>
<td>Calcium chloride dihydrate</td>
<td>219.000</td>
</tr>
<tr>
<td>Magnesium sulphate anhydrous</td>
<td>97.720</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>330.000</td>
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<tr>
<td>Potassium nitrate</td>
<td>0.076</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>4505.000</td>
</tr>
<tr>
<td>Sodium dihydrogen phosphate anhydrous</td>
<td>109.000</td>
</tr>
<tr>
<td>Sodium selenite</td>
<td>0.0173</td>
</tr>
<tr>
<td><strong>AMINO ACIDS</strong></td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>30.000</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>25.000</td>
</tr>
<tr>
<td>L-Arginine hydrochloride</td>
<td>84.000</td>
</tr>
<tr>
<td>L-Asparagine</td>
<td>25.000</td>
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<tr>
<td>L-Aspartic acid</td>
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<tr>
<td>L-Cystine dihydrochloride</td>
<td>91.240</td>
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<tr>
<td>L-Glutamic acid</td>
<td>75.000</td>
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<tr>
<td>L-Histidine hydrochloride monohydrate</td>
<td>42.000</td>
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<tr>
<td>L-Isoleucine</td>
<td>104.800</td>
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<tr>
<td>L-Leucine</td>
<td>104.800</td>
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<tr>
<td>L-Lysine hydrochloride</td>
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<tr>
<td>L-Methionine</td>
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<tr>
<td>L-Phenylalanine</td>
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<td>L-Proline</td>
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<td>L-Serine</td>
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<tr>
<td>L-Threonine</td>
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<tr>
<td>L-Tryptophan</td>
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<tr>
<td>L-Tyrosine disodium salt</td>
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<tr>
<td>L-Valine</td>
<td>93.600</td>
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<tr>
<td><strong>VITAMINS</strong></td>
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<tr>
<td>Choline chloride</td>
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<tr>
<td>D-Biotin</td>
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<tr>
<td>D-Ca-Pantothenate</td>
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<tr>
<td>Folic acid</td>
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<td>Nicotinamide</td>
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<tr>
<td>Pyridoxal hydrochloride</td>
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<tr>
<td>Riboflavin</td>
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<tr>
<td>Thiamine hydrochloride</td>
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<td>Vitamin B12</td>
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<tr>
<td>i-Inositol</td>
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<tr>
<td><strong>OTHERS</strong></td>
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<tr>
<td>D-Glucose</td>
<td>4500.000</td>
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<tr>
<td>HEPES Buffer</td>
<td>5958.000</td>
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<tr>
<td>Phenol red sodium salt</td>
<td>15.000</td>
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<tr>
<td>Sodium pyruvate</td>
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</table>

Directions:

1. Suspend 17.1gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 3.024gms of sodium bicarbonate powder (TC230) or 40.32ml of 7.5% sodium bicarbonate solution (TCL013) and 0.584gms of L-glutamine powder (TC243) or 20.0ml of 200mM L-glutamine solution (TCL012) for 1 litre of medium and stir until dissolved.
3. Adjust the pH to 0.2-0.3 pH units below the desired pH using 1N HCl or 1N NaOH since the pH tends to rise during filtration.
4. Make up the final volume to 1000ml with tissue culture grade water.
5. Sterilize the medium immediately by filtering through a sterile membrane filter with a porosity of 0.22 micron or less, using positive pressure rather than vacuum to minimize the loss of carbon dioxide.
6. Aseptically add sterile supplements as required and dispense the desired amount of sterile medium into sterile containers.
7. Store liquid medium at 2-8°C and in dark till use.

**Material required but not provided:**
- Tissue culture grade water (TCL010)
- Sodium bicarbonate (TC230)
- Sodium bicarbonate solution, 7.5% (TCL013)
- L-Glutamine powder (TC243)
- L-Glutamine solution 200mM (TCL012)
- 1N Hydrochloric acid (TCL003)
- 1N Sodium hydroxide (TCL002)
- Foetal bovine serum (RM1112/RM10432)

**Quality Control:**

**Appearance**
Off-white to Creamish white, homogenous powder.

**Solubility**
Clear solution at 17.1 gms/L.

**pH without Sodium Bicarbonate**
4.60 - 5.20

**pH with Sodium Bicarbonate**
6.70 - 7.30

**Osmolality without Sodium Bicarbonate**
210.00 - 250.00

**Osmolality with Sodium Bicarbonate**
280.00 - 320.00

**Cultural Response**
The growth promotion capacity of the medium is assessed qualitatively by analyzing the cells for the morphology and quantitatively by estimating the cell counts and comparing it with a control medium through minimum three subcultures.

**Endotoxin Content**
NMT 5EU/ml

**Storage and Shelf Life:**
1. All the powdered media and prepared liquid culture media should be stored at 2-8°C. Use before the expiry date. In spite of above recommended storage condition, certain powdered medium may show some signs of deterioration /degradation in certain instances. This can be indicated by change in colour, change in appearance and presence of particulate matter and haziness after dissolution.
2. Preparation of concentrated medium is not recommended since free base amino acids and salt complexes having low solubility may precipitate in concentrated medium.
3. pH and sodium bicarbonate concentration of the prepared medium are critical factors affecting cell growth. This is also influenced by amount of medium and volume of culture vessel used (surface to volume ratio). For example, in large bottles, such as Roux bottles pH tends to rise perceptibly as significant volume of carbon dioxide is released. Therefore, optimal conditions of pH, sodium bicarbonate concentration, surface to volume ratio must be determined for each cell type. We recommend stringent monitoring of pH. If needed, pH can be adjusted by using sterile 1N HCl or 1N NaOH or by bubbling in carbon dioxide.
4. If required, supplements can be added to the medium prior to or after filter sterilization observing sterility precautions. Shelf life of the medium will depend on the nature of supplement added to the medium.

**Disclaimer:**
User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

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