

## 1494: . OMIZ-PAT Medium (modified)

Final pH: 6.9

Final volume: 200 ml

|  |        |    |
|--|--------|----|
| Distilled water                                    | 100.00 | ml |
| <b>Solution A</b>                                  | 10.00  | ml |
| <b>Solution B</b>                                  | 10.00  | ml |
| <b>Solution C</b>                                  | 10.00  | ml |
| <b>Solution D</b>                                  | 10.00  | ml |
| <b>Solution E</b>                                  | 10.00  | ml |
| <b>Solution F</b>                                  | 10.00  | ml |
| <b>Solution G</b>                                  | 10.00  | ml |
| <b>Solution H</b>                                  | 200.00 | µl |
| <b>Solution I</b>                                  | 8.00   | µl |
| <b>Solution J</b>                                  | 2.00   | µl |
| <b>Solution K</b>                                  | 40.00  | µl |
| <b>Solution L</b>                                  | 2.00   | ml |
| <b>Trace elements I (pre-dilute 1:1000; use)</b>   | 2.00   | ml |
| <b>Trace elements II (pre-dilute 1:1000; use)</b>  | 2.00   | ml |
| <b>Trace elements III (pre-dilute 1:1000; use)</b> | 200.00 | µl |
| Distilled water (add up to 200 ml)                 | 200.00 | ml |
| <b>Supplement 1</b>                                | 200.00 | µl |
| <b>Supplement 2</b>                                | 2.00   | ml |
| <b>Supplement 3</b>                                | 2.00   | ml |
| <b>Supplement 4</b>                                | 200.00 | µl |
| <b>Supplement 5</b>                                | 2.00   | ml |

1. Add 10 ml rabbit serum, flush the medium with N<sub>2</sub>CO<sub>2</sub>, adjust the pH to 6.9 and filter-sterilize the completed medium into tubes.

|              |       |    |
|--------------|-------|----|
| Rabbit serum | 10.00 | ml |
|--------------|-------|----|

2. This recipe describes the preparation of 200 ml medium, which can be used for about 4 weeks when kept at 4°C.

### Solution A

|                 |         |    |
|-----------------|---------|----|
| L-Alanine       | 90.00   | mg |
| L-Arginine      | 350.00  | mg |
| L-Asparagine    | 300.00  | mg |
| L-Aspartic acid | 236.00  | mg |
| L-Cysteine HCl  | 700.00  | mg |
| L-Glutamine     | 1360.00 | mg |

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|-----------------|---------|----|
| L-Glutamic acid | 600.00  | mg |
| Glycine         | 150.00  | mg |
| L-Histidine     | 1240.00 | mg |
| L-Isoleucine    | 262.00  | mg |
| L-Leucine       | 262.00  | mg |
| L-Lysine HCl    | 364.00  | mg |
| L-Methionine    | 300.00  | mg |
| L-Ornithine HCl | 340.00  | mg |
| L-Phenylalanine | 330.00  | mg |
| L-Proline       | 230.00  | mg |
| L-Serine        | 1050.00 | mg |
| L-Threonine     | 240.00  | mg |
| L-Tryptophan    | 204.00  | mg |
| L-Tyrosine      | 180.00  | mg |
| L-Valine        | 234.00  | mg |
| Distilled water | 100.00  | ml |

Prepare Solutions A, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

### Solution B

|   |         |    |
|---|---------|----|
| KCl   | 1936.00 | mg |
| MgSO <sub>4</sub> × 7 H <sub>2</sub> O              | 500.00  | mg |
| NaH <sub>2</sub> PO <sub>4</sub> × H <sub>2</sub> O | 280.00  | mg |
| NH <sub>4</sub> Cl                                  | 3200.00 | mg |
| Citric acid, trisodium salt                         | 400.00  | mg |
| Distilled water                                     | 100.00  | ml |

Prepare Solutions B, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

### Solution C

|  |        |    |
|--|--------|----|
| Calcium D-(+)-pantothenate                       | 10.00  | mg |
| Choline chloride                                 | 100.00 | mg |
| Myo-inositol                                     | 100.00 | mg |
| Thiamine HCl                                     | 10.00  | mg |
| Thiamin pyrophosphate                            | 10.00  | mg |
| Coenzym A, sodium salt                           | 2.00   | mg |
| Flavin adenine dinucleotide (FAD)                | 2.00   | mg |
| β-nicotinamide adenine dinucleotide (NAD)        | 2.00   | mg |
| 2-Mercaptoethanesulfonic acid (MES), sodium salt | 20.00  | mg |
| N-acetylmuramic acid                             | 50.00  | mg |
| N-Acetylglucosamine                              | 400.00 | mg |

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|                                |         |    |
|--------------------------------|---------|----|
| D-glucuronic acid, sodium salt | 1600.00 | mg |
| D-galacturonic acid            | 1600.00 | mg |
| Pyruvic acid, sodium salt      | 1100.00 | mg |
| Fumaric acid, disodium salt    | 1000.00 | mg |
| Formic acid, sodium salt       | 600.00  | mg |
| Distilled water                | 100.00  | ml |

Prepare Solutions C, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

### Solution D

|  |         |    |
|--|---------|----|
| Pyridoxal hydrochloride                    | 10.00   | mg |
| Pyridoxal phosphate                        | 10.00   | mg |
| D, L-lactic acid, sodium salt              | 1120.00 | mg |
| Hypoxanthine                               | 2.80    | mg |
| Uracil                                     | 2.20    | mg |
| Thymidine                                  | 4.80    | mg |
| N-(2-acetamido)-2-aminoethanesulfonic acid | 3.60    | mg |
| DL-carnitine                               | 400.00  | mg |
| Putrescine 2 HCl                           | 10.00   | mg |
| Phenol red, sodium salt                    | 20.00   | mg |
| Distilled water                            | 100.00  | ml |

Prepare Solutions D, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

### Solution E

|                 |        |    |
|-----------------|--------|----|
| D-Glucose       | 4.00   | g  |
| D-Fructose      | 1.60   | g  |
| D-Maltose       | 1.60   | g  |
| D-Mannitol      | 1.60   | g  |
| D-Mannose       | 4.00   | g  |
| D-Arabinose     | 4.00   | g  |
| L-Fucose        | 4.00   | g  |
| D-Trehalose     | 4.00   | g  |
| D-Sucrose       | 4.00   | g  |
| L-Rhamnose      | 4.00   | g  |
| Distilled water | 100.00 | ml |

Prepare Solutions E, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

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### Solution F

|                                      |        |    |
|--------------------------------------|--------|----|
| CaCl <sub>2</sub> x H <sub>2</sub> O | 300.00 | mg |
| Distilled water                      | 100.00 | ml |

Prepare Solution F, flush with nitrogen to make it anoxic, filter-sterilize and store at 4°C

### Solution G

|              |      |       |
|--------------|------|-------|
| Asialofetuin | 1.00 | mg/ml |
|--------------|------|-------|

Prepare Solutions G, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

### Solution H

|                            |        |    |
|----------------------------|--------|----|
| Vitamine B <sub>12</sub>   | 5.00   | mg |
| D-(+)-biotin               | 5.00   | mg |
| Folic acid                 | 5.00   | mg |
| Folinic acid, calcium salt | 10.00  | mg |
| Nicotinamide               | 5.00   | mg |
| Nicotinic acid             | 10.00  | mg |
| Riboflavine                | 1.00   | mg |
| Distilled water            | 100.00 | ml |

Prepare Solutions H, flush each with nitrogen to make them anoxic, filter-sterilize and store at -20°C

### Solution I

|                      |       |    |
|----------------------|-------|----|
| Isobutyric acid      | 10.00 | μl |
| 2-Methylbutyric acid | 10.00 | μl |
| Valeric acid         | 10.00 | μl |
| Isovaleric acid      | 10.00 | μl |

Prepare Solution I and store at -20°C

### Solution J

|                      |      |       |
|----------------------|------|-------|
| DL-alpha-lipoic acid | 1.00 | mg/ml |
| 2-Mercaptoethanol    | 0.10 | ml    |
| Ethanol              | 0.90 | ml    |

D,L-alpha-lipoic acid (1 mg/ml); in 2-mercaptoethanol-ethanol (1:10); store at -20°C

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### Solution K

|       |       |    |
|-------|-------|----|
| Hemin | 0.20  | mM |
| NaOH  | 10.00 | mM |

Hemin (0.2 mM) in NaOH (10 mM)

### Solution L

|                       |        |    |
|-----------------------|--------|----|
| Yeast extract (fresh) | 42.00  | g  |
| Distilled water       | 140.00 | ml |

Homogenize 42 g fresh baker yeast in 140 ml distilled water, incubate for 24h at 56°C and centrifuge to harvest the supernatant. Adjust pH to 7 with NaOH, make anoxic, filter-sterilize and store at -80°C

### Trace elements I

|  |        |    |
|--|--------|----|
| CuSO <sub>4</sub>                      | 7.89   | mg |
| MnSO <sub>4</sub> x H <sub>2</sub> O   | 169.00 | mg |
| ZnSO <sub>4</sub> x 7 H <sub>2</sub> O | 2.87   | g  |
| HCl (10 mM)                            | 100.00 | ml |

Prepare Trace elements I, flush with nitrogen to make them anoxic, filter-sterilize and store at 4°C

### Trace elements II

|                                  |        |    |
|----------------------------------|--------|----|
| Na <sub>2</sub> SeO <sub>3</sub> | 173.00 | mg |
| HCl (10mM)                       | 100.00 | ml |

Prepare Trace elements II, flush with nitrogen to make them anoxic, filter-sterilize and store at 4°C

### Trace elements III

|  |         |    |
|--|---------|----|
| NiSO <sub>4</sub> x 6 H <sub>2</sub> O   | 13.10   | mg |
| SnCl <sub>2</sub> x 2 H <sub>2</sub> O   | 11.80   | mg |
| NaVO <sub>3</sub>  | 61.00   | mg |
| (NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> x 4 H <sub>2</sub> O | 1240.00 | mg |
| HCl (10 mM)  | 100.00  | ml |

Prepare Trace elements III, flush with nitrogen to make them anoxic, filter-sterilize and store at 4°C

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### Supplement 1

|   |       |    |
|---|-------|----|
| Fe SO <sub>4</sub> x 7 H <sub>2</sub> O | 10.00 | mM |
| HCl                                     | 10.00 | mM |

1. FeSO<sub>4</sub> x 7H<sub>2</sub>O (10 mM) in HCl (10 mM)
2. Prepare Supplements 1, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

### Supplement 2

|                 |       |        |
|-----------------|-------|--------|
| Ascorbic acid   | 10.00 | %(w/v) |
| Distilled water | 2.00  | ml     |

1. Ascorbic acid (10%; w/v); in distilled water, neutralize with NaOH
2. Prepare Supplements 2, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

### Supplement 3

|                 |      |    |
|-----------------|------|----|
| Glutathione     | 0.50 | M  |
| Distilled water | 2.00 | ml |

1. Glutathion (0.5 M); in distilled water, neutralize with NaOH
2. Prepare Supplements 3, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

### Supplement 4

|                 |      |       |
|-----------------|------|-------|
| Cholesterol     | 1.00 | mg/ml |
| Ethanol (100 %) | 1.00 | ml    |

Cholesterol (1 mg/ml); in Ethanol (100%); store at -20°C

### Supplement 5

|                    |       |        |
|--------------------|-------|--------|
| NaHCO <sub>3</sub> | 10.00 | %(w/v) |
| Distilled water    | 2.00  | ml     |

NaHCO<sub>3</sub> (10%; w/v) in distilled water, store at 4°C