**Extended Data**

**Figure 1-1. Composition of holidic diet media.**

|  |  |  |
| --- | --- | --- |
| Components | Stocks | Diets |
| Before autoclaving |  | **25N** | **50N** | **200N** | **400N** |
| Agar (g) | - | 20 | 20 | 20 | 20 |
| L- Isoleucine (g) | - | 0.2275 | 0.455 | 1.82 | 3.64 |
| L- Leucine (g) | - | 0.1512 | 0.3025 | 1.21 | 2.42 |
| L- Tyrosine (g)  | - | 0.0525 | 0.105 | 0.42 | 0.84 |
| Sucrose (g) | - | 17.12 | 17.12 | 17.12 | 17.12 |
| Base Buffer (ml)\* | 10x | 100 | 100 | 100 | 100 |
| Calcium Chloride (ml) | 250 g/L | 1 | 1 | 1 | 1 |
| Magnesium Sulphate (ml) | 250 g/L | 1 | 1 | 1 | 1 |
| Copper Sulphate (ml) | 2.5 g/L | 1 | 1 | 1 | 1 |
| Iron Sulphate (ml) | 25 g/L | 1 | 1 | 1 | 1 |
| Manganese Chloride (ml) | 1 g/L | 1 | 1 | 1 | 1 |
| Zinc Sulphate (ml) | 25 g/L | 1 | 1 | 1 | 1 |
| Cholesterol (ml)\* | 20 mg/mL | 15 | 15 | 15 | 15 |
|  |  |  |  |  |  |
| After autoclaving |  |  |  |  |  |
| Nucleic acids/Lipids (ml)\* | 125x | 8 | 8 | 8 | 8 |
| Essential amino acids (ml)\* |  | 7.564 | 15.128 | 60.512 | 121.02 |
| Non-essential amino acids (ml)\* |  | 7.564 | 15.128 | 60.512 | 121.02 |
| Sodium Glutamate (ml) | 100 g/L | 1.89 | 3.78 | 15.12 | 30.24 |
| Vitamin (ml)\* | 47.6x | 21 | 21 | 21 | 21 |
| Folic Acid (ml) | 0.5 g/L | 1 | 1 | 1 | 1 |
| Propionic Acid (ml) | - | 6 | 6 | 6 | 6 |
| Nipagin (ml)\* | 100 g/L | 15 | 15 | 15 | 15 |

\* Refer to Piper et al., 2014 for complete composition

**Figure 6-1. Composition of 25N-F/H/W holidic medium.**

|  |  |  |
| --- | --- | --- |
| Components | Stocks | Diets |
| Before autoclaving |  | **25N-F/H/W** |
| Agar (g) | - | 20 |
| L- Isoleucine (g) | - | 0.455 |
| L- Leucine (g) | - | 0.3025 |
| L- Tyrosine (g)  | - | 0.105 |
| Sucrose (g) | - | 17.12 |
| Base Buffer (ml)\* | 10x | 100 |
| Calcium Chloride (ml) | 250 g/L | 1 |
| Magnesium Sulphate (ml) | 250 g/L | 1 |
| Copper Sulphate (ml) | 2.5 g/L | 1 |
| Iron Sulphate (ml) | 25 g/L | 1 |
| Manganese Chloride (ml) | 1 g/L | 1 |
| Zinc Sulphate (ml) | 25 g/L | 1 |
| Cholesterol (ml)\* | 20 mg/mL | 15 |
|  |  |  |
| After autoclaving |  |  |
| Nucleic acids/Lipids (ml)\* | 125x | 8 |
| Essential amino acids (ml)\* (excluding Phe, His, Trp) |  | 15.128 |
| Phe, His, Trp solution |  | 7.564 |
| Non-essential amino acids (ml)\* |  | 15.128 |
| Sodium Glutamate (ml) | 100 g/L | 3.78 |
| Vitamin (ml)\* | 47.6x | 21 |
| Folic Acid (ml) | 0.5 g/L | 1 |
| Propionic Acid (ml) | - | 6 |
| Nipagin (ml)\* | 100 g/L | 15 |

\* Refer to Piper et al., 2014 for complete composition

**Figure 7-1. Composition of Neurobasal-based medium minus phenol red and amino acids.**

|  |  |  |
| --- | --- | --- |
| Components | Concentration (mg/L) | mM |
| Vitamins |  |  |
| Choline chloride | 4 | 0.02857143 |
| D-Calcium pantothenate | 4 | 0.00838574 |
| Folic Acid | 4 | 0.0090703 |
| Niacinamide | 4 | 0.03278688 |
| Pyridoxal hydrochloride | 4 | 0.01960784 |
| Riboflavin | 0.4 | 0.00106383 |
| Thiamine hydrochloride | 4 | 0.01186944 |
| Vitamin B12 | 0.0068 | 5.02E-06 |
| i-Inositol | 7.2 | 0.04 |
| Inorganic Salts |  |  |
| Calcium Chloride (CaCl2) (anhyd.) | 200 | 1.8018018 |
| Ferric Nitrate (Fe(NO3)3"9H2O) | 0.1 | 2.48E-04 |
| Magnesium Chloride (anhydrous) | 77.3 | 0.8136842 |
| Potassium Chloride (KCl) | 400 | 5.3333335 |
| Sodium Bicarbonate (NaHCO3) | 2200 | 26.190475 |
| Sodium Chloride (NaCl) | 3000 | 51.724136 |
| Sodium Phosphate monobasic (NaH2PO4-H2O) | 125 | 0.9057971 |
| Zinc sulfate (ZnSO4-7H2O) | 0.194 | 6.74E-04 |
| Other components |  |  |
| D-Glucose (Dextrose) | 4500 | 25 |
| HEPES | 2600 | 10.92437 |
| Phenol Red | 8.1 | 0.02151966 |
| Sodium Pyruvate | 25 | 0.22727273 |