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| **CFB-PROCEDURE** | **Document** | **BABER0003** |
| ***Strain tolerance screening***  ***Media preparation*** | **Revision no** | **Draft 3** |
| **Revision data** | **2015-03-02** |
| **Author** | **BABER** |
| **Pages** | **1 of 13** |

**STOCK SOLUTIONS**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Compound** | **Formula** | **Mw (g/mol)** | **Concentration (g/L)** | **Concentration (M)** | **Volume (mL)** | **Amount** |
| 22.5X Glucose | C6H12O6 | 180,2 | 450 | 2,5 | 150,0 | 180,0 g |
| 1.47X Glucose | C6H12O6 | 180,2 | 390,34 | 2,17 | 169,7 | 66,4 g |
| 1.47X Sucrose | C12H22O11 | 342,3 | 390,34 | 1,14 | 169,7 | 66,4 g |
| 50% (v/v) Glycerol | C3H8O3 | 92,09 | 630,5 | 6,85 | 100,0 | 50,0 mL |
| 50% (v/v) Ethanol | C2H5OH | 46,07 | 394,5 | 8,6 | 100,0 | 50,0 mL |
| Ethanol 99% \* | C2H5OH | 46,07 | 781,11 | 17,0 | 30 |  |
| Acetic acid (glacial) \* | CH3CO2H | 60,05 | 1049 | 17,5 | 2,5 |  |
| 1,4-butanediol \* | C4H10O2 | 90,12 | 1017,1 | 11,29 | 15 |  |
| R-(+)-Limonene \* | C10H16 | 136,23 | 815,8 | 5,99 | 7 |  |
| Formic acid \* | CH2O2 | 46,03 | 1220 | 26,5 | 1 |  |
| Furfural \* | C5H4O2 | 96,08 | 1160,0 | 12,07 | 1 |  |
| Trace elements 500X | - | - | - | - | 22 |  |
| Vitamins 1000X | - | - | - | - | 11 |  |

\* These are concentrated liquids with the given concentrations.

**5X BUFFER SOLUTIONS**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Compound** | **Mw** | **Concentration (g/L)** | **Concentration (mM)** | **Final volume (mL)** | **Amount (g)** |
| Maleic acid  (pKa = 1.9, 6.07) | 116,07 | 29,02 | 250,0 | 100,0 | 2,902 |
| L-(+)-Tartaric acid  (pKa = 2.98) | 150,09 | 37,52 | 250,0 | 500,0 | 18,761 |
| Ammonium tartrate dibasic (pKa = 2.95, 4.25) | 184,15 | 46,04 | 250,0 | 100,0 | 4,604 |
| Potassium hydrogen phthalate (pKa = 5.4) | 204,22 | 51,06 | 250,0 | 500,0 | 25,528 |
| BES (pKa = 7.1) | 213,25 | 53,31 | 250,0 | 100,0 | 5,331 |
| CAPS (pKa = 10.4) | 221,32 | 55,33 | 250,0 | 100,0 | 5,533 |

**1.18X BUFFER SOLUTIONS**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Compound** | **Mw** | **Concentration (g/L)** | **Concentration (mM)** | **Final volume (mL)** | **Amount (g)** |
| L-(+)-Tartaric acid  (pKa = 2.98) | 150,09 | 9,51 | 63,4 | 845,3 | 8,037 |
| Potassium hydrogen phthalate (pKa = 5.4) | 204,22 | 12,94 | 63,4 | 845,3 | 10,936 |

**10X SALT SOLUTIONS**

**10X Salts A + Vitamins + Trace (pH 2/3/7/10.5)**

**Final volume: 500 mL**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Compound** | **Formula** | **Mw** | **Concentration**  **(g/L)** | **Concentration**  **(mM)** | **Amount**  **(g)** |
| Ammonium sulfate | (NH4)2SO4 | 132,14 | 66,07 | 500,0 | 33,035 |
| Potassium phosphate monobasic | KH2PO4 | 136,09 | 68,05 | 500,0 | 34,023 |
| Magnesium sulfate heptahydrate | MgSO4·7 H2O | 246,47 | 5,00 | 20,30 | 2,502 |
| **Compound** |  |  | **Concentration**  **(mL/L)** |  | **Amount**  **(mL)** |
| 500X Trace elements |  |  | 20 |  | 10,000 |
| 1000X Vitamins |  |  | 10 |  | 5,000 |

**10X Salts B + Vitamins + Trace (pH 5.5)**

**Final volume: 500 mL**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Compound** | **Formula** | **Mw** | **Concentration**  **(g/L)** | **Concentration**  **(mM)** | **Amount**  **(g)** |
| Ammonium sulfate | (NH4)2SO4 | 132,14 | 66,07 | 500,0 | 33,035 |
| Sodium phosphate monobasic | NaH2PO4 | 119,98 | 59,99 | 500,0 | 29,995 |
| Magnesium sulfate heptahydrate | MgSO4·7 H2O | 246,47 | 5,00 | 20,30 | 2,502 |
| **Compound** |  |  | **Concentration**  **(mL/L)** |  | **Amount**  **(mL)** |
| 500X Trace elements |  |  | 20 |  | 10,000 |
| 1000X Vitamins |  |  | 10 |  | 5,000 |

**10X Salts C + Vitamins + Trace (pH 4.5)**

**Final volume: 100 mL**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Compound** | **Formula** | **Mw** | **Concentration**  **(g/L)** | **Concentration**  **(mM)** | **Amount**  **(g)** |
| Sodium sulfate | Na2SO4 | 142,04 | 71,02 | 500,0 | 7,102 |
| Potassium phosphate monobasic | KH2PO4 | 136,09 | 68,05 | 500,0 | 6,805 |
| Magnesium sulfate heptahydrate | MgSO4·7 H2O | 246,47 | 5,00 | 20,30 | 0,500 |
| **Compound** |  |  | **Concentration**  **(mL/L)** |  | **Amount**  **(mL)** |
| 500X Trace elements |  |  | 20 |  | 2,000 |
| 1000X Vitamins |  |  | 10 |  | 1,000 |

**MEDIA WITH DIFFERENT pH, 2% glucose**

**21.43 g/L glucose (1.071X ), pH 2, 7.0, 10.5**

**Final volume: 50 mL**

|  |  |  |  |
| --- | --- | --- | --- |
| **Stock solution** | **Concentration** | **Final concentration** | **Amount (mL)** |
| 5X MaleicAc Buffer pH 2  5X BES Buffer pH 7.0  5X CAPS Buffer pH 10.5 | 5X | 1.071X | 10,71 |
| 10X Salts A (pH 2/3/7/10.5) | 10X | 1.071X | 5,355 |
| 450 g/L Glucose | 1,47X | 1X | 2,380 |
| Water |  |  | 31,555 |

**21.43 g/L glucose (1.071X ), pH 4.5**

**Final volume: 50 mL**

|  |  |  |  |
| --- | --- | --- | --- |
| **Stock solution** | **Concentration** | **Final concentration** | **Amount (mL)** |
| 5X AmmTar Buffer pH 4.5 | 5X | 1.071X | 10,71 |
| 10X Salts C (pH 4.5) | 10X | 1.071X | 5,355 |
| 450 g/L Glucose | 1,47X | 1X | 2,380 |
| Water |  |  | 31,555 |

**21.43 g/L glucose (1.071X ), pH 3**

**Final volume: 1000 mL**

|  |  |  |  |
| --- | --- | --- | --- |
| **Stock solution** | **Concentration** | **Final concentration** | **Amount (mL)** |
| 1.18X TartarAc Buffer pH 3 | 1.18X | 1X | 845,3 |
| 10X Salts A (pH 2/3/7/10.5) | 10X | 1.071X | 107,1 |
| 450 g/L Glucose | 1,47X | 1X | 47,6 |

**21.43 g/L glucose (1.071X ), pH 5.5**

**Final volume: 1000 mL**

|  |  |  |  |
| --- | --- | --- | --- |
| **Stock solution** | **Concentration** | **Final concentration** | **Amount (mL)** |
| 1.18X KHP Buffer pH 5.5 | 1.18X | 1X | 845,3 |
| 10X Salts B (pH 5.5) | 10X | 1.071X | 107,1 |
| 450 g/L Glucose | 1,47X | 1X | 47,6 |

**MEDIA WITH DIFFERENT INHIBITORS, pH 5.5**

**21.43 g/L glucose + 150 g/L Ethanol (1.071X ), pH 5.5**

**Final volume (mL): 50.0**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Solution** | **Formula** | **Mw** | **Stock concentration (X)** | **Final Concentration (g/L)** | **Final Concentration (mL/L)** | **Amount (mL)** |
| 10X Salts + Vitamins + Trace (pH 5.5) |  |  | 10 |  | 107.1 | 5.355 |
| 51.06 g/L KHP Buffer pH 5.5 | HOOCC6H4COOK | 204.22 | 5 | 10.94 | 214.2 | 10.71 |
| Ethanol 99% (789 g/L) | C2H5OH | 46.07 | 5.3 | 150.0 | 190.11 | 9.506 |
| Water |  |  |  |  |  | 24.429 |
| **Compound** | **Formula** | **Mw** | **Stock concentration (g/L)** | **Final Concentration (g/L)** | **Final Concentration (mM)** | **Amount (g)** |
| Glucose | C6H12O6 | 180.16 |  | 21.42 | 118.89 | 1.071 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Medium** | **Final concentration after inoculation (g/L)** | **Working concentration (g/L)** | **Final volume (mL)** | **Concentrated medium (g/L)** | **Concentrated medium (mL)** | **21.43 g/L Glucose, pH 5.5 (1.071X) (mL)** |
| 2% glucose, 1.1% Ethanol, pH 5.5 | 11.36 | 12.17 | 15.0 | 150.0 | 1.217 | 13.783 |
| 2% glucose, 2.5% Ethanol, pH 5.5 | 25.00 | 26.78 | 15.0 | 150.0 | 2.678 | 12.323 |
| 2% glucose, 4.5% Ethanol, pH 5.5 | 45.00 | 48.20 | 15.0 | 150.0 | 4.820 | 10.181 |
| 2% glucose, 6.5% Ethanol, pH 5.5 | 65.00 | 69.62 | 15.0 | 150.0 | 6.962 | 8.039 |
| 2% glucose, 7.9% Ethanol, pH 5.5 | 78.66 | 84.25 | 15.0 | 150.0 | 8.425 | 6.575 |
|  |  |  |  | **SUM:** | **24.10** | **50.90** |

**Procedure**

1. Mix all liquid solutions in Table 1 in a 50 mL Falcon tube to prepare a concentrated stock solution. Work in a LAF-bench to keep stock solutions sterile.
2. Bring the mixture to the chemical room and add the solid compounds in Table 1 and adjust the pH with either NaOH or Tartaric acid.
3. Bring the solution back to the LAF-bench, complete the volume to 50 mL and filter sterilize into a new tube.
4. Prepare the number of tubes indicated in Table 2 by labelling with corresponding concentration. Transfer the indicated volume into each tube.
5. Complete the volume to 15 mL by adding buffered minimal media with glucose.
6. Each solution of 15 mL is enough for 42 wells using 280 µL per well in a 96-well plate.

**21.43 g/L glucose + 250 g/L 1,4-Butanediol, pH 5.5**

**Final volume (mL): 50**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Solution** | **Formula** | **Mw** | **Stock concentration (X)** | **Final Concentration (g/L)** | **Final Concentration (mL/L)** | **Amount (mL)** |
| 10X Salts + Vitamins + Trace (pH 5.5) |  |  | 10 |  | 107.1 | 5.355 |
| 51.06 g/L KHP Buffer pH 5.5 | HOOCC6H4COOK | 204.22 | 5 | 10.94 | 214.2 | 10.71 |
| 1,4-butanediol (1017.1 g/L) | C4H10O2 | 90.12 | 4.1 | 250.0 | 245.80 | 12.290 |
| Water |  |  |  |  |  | 21.645 |
| **Compound** | **Formula** | **Mw** | **Stock concentration (g/L)** | **Final Concentration (g/L)** | **Final Concentration (mM)** | **Amount (g)** |
| Glucose | C6H12O6 | 180.16 |  | 21.42 | 118.89 | 1.071 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Medium** | **Final concentration after inoculation (g/L)** | **Working concentration (g/L)** | **Final volume (mL)** | **Concentrated medium (g/L)** | **Concentrated medium (mL)** | **21.43 g/L Glucose, pH 5.5 (1.071X) (mL)** |
| 2% glucose, 5.3% 1,4-Butanediol, pH 5.5 | 53.20 | 56.98 | 15.0 | 250.0 | 3.419 | 11.581 |
| 2% glucose, 6% 1,4-Butanediol, pH 5.5 | 60.00 | 64.26 | 15.0 | 250.0 | 3.856 | 11.144 |
| 2% glucose, 7% 1,4-Butanediol, pH 5.5 | 70.00 | 74.97 | 15.0 | 250.0 | 4.498 | 10.502 |
| 2% glucose, 8% 1,4-Butanediol, pH 5.5 | 80.00 | 85.68 | 15.0 | 250.0 | 5.141 | 9.859 |
| 2% glucose, 8.7% 1,4-Butanediol, pH 5.5 | 86.80 | 92.96 | 15.0 | 250.0 | 5.578 | 9.422 |
|  |  |  |  | **SUM:** | **22.49** | **52.51** |

**Procedure**

1. Mix all liquid solutions in Table 1 in a 50 mL Falcon tube to prepare a concentrated stock solution. Work in a LAF-bench to keep stock solutions sterile.
2. Bring the mixture to the chemical room and add the solid compounds in Table 1 and adjust the pH with either NaOH or Tartaric acid.
3. Bring the solution back to the LAF-bench, complete the volume to 50 mL and filter sterilize into a new tube.
4. Prepare the number of tubes indicated in Table 2 by labelling with corresponding concentration. Transfer the indicated volume into each tube.
5. Complete the volume to 15 mL by adding buffered minimal media with glucose.
6. Each solution of 15 mL is enough for 42 wells using 280 µL per well in a 96-well plate.

**21.43 g/L glucose + 1.7 g/L PABA, pH 5.5**

**Final volume (mL): 50**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Solution** | **Formula** | **Mw** | **Stock concentration (X)** | **Final Concentration (g/L)** | **Final Concentration (mL/L)** | **Amount (mL)** |
| 10X Salts + Vitamins + Trace (pH 5.5) |  |  | 10 |  | 107.1 | 5.355 |
| 51.06 g/L KHP Buffer pH 5.5 | HOOCC6H4COOK | 204.22 | 5 | 10.94 | 214.2 | 10.71 |
| Water |  |  |  |  |  | 33.935 |
| **Compound** | **Formula** | **Mw** | **Stock concentration (g/L)** | **Final Concentration (g/L)** | **Final Concentration (mM)** | **Amount (g)** |
| Glucose | C6H12O6 | 180.16 |  | 21.42 | 118.89 | 1.071 |
| 4-Aminobenzoic acid | C7H7NO2 | 137.14 |  | 1.70 | 12.40 | 0.085 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Medium** | **Final concentration after inoculation (g/L)** | **Working concentration (g/L)** | **Final volume (mL)** | **Concentrated medium (g/L)** | **Concentrated medium (mL)** | **21.43 g/L Glucose, pH 5.5 (1.071X) (mL)** |
| 2% glucose, 0.008% PABA, pH 5.5 | 0.08 | 0.09 | 15.0 | 1.70 | 0.756 | 14.244 |
| 2% glucose, 0.025% PABA, pH 5.5 | 0.25 | 0.27 | 15.0 | 1.70 | 2.363 | 12.638 |
| 2% glucose, 0.050% PABA, pH 5.5 | 0.50 | 0.54 | 15.0 | 1.70 | 4.725 | 10.275 |
| 2% glucose, 0.075% PABA, pH 5.5 | 0.75 | 0.80 | 15.0 | 1.70 | 7.088 | 7.913 |
| 2% glucose, 0.092% PABA, pH 5.5 | 0.92 | 0.99 | 15.0 | 1.70 | 8.694 | 6.306 |
|  |  |  |  | **SUM:** | **23.63** | **51.38** |

**Procedure**

1. Mix all liquid solutions in Table 1 in a 50 mL Falcon tube to prepare a concentrated stock solution. Work in a LAF-bench to keep stock solutions sterile.
2. Bring the mixture to the chemical room and add the solid compounds in Table 1 and adjust the pH with either NaOH or Tartaric acid.
3. Bring the solution back to the LAF-bench, complete the volume to 50 mL and filter sterilize into a new tube.
4. Prepare the number of tubes indicated in Table 2 by labelling with corresponding concentration. Transfer the indicated volume into each tube.
5. Complete the volume to 15 mL by adding buffered minimal media with glucose.
6. Each solution of 15 mL is enough for 42 wells using 280 µL per well in a 96-well plate.

**21.43 g/L glucose + 100 g/L D-Limonene, pH 5.5**

**Final volume (mL): 50**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Solution** | **Formula** | **Mw** | **Stock concentration (X)** | **Final Concentration (g/L)** | **Final Concentration (mL/L)** | **Amount (mL)** |
| 10X Salts + Vitamins + Trace (pH 5.5) |  |  | 10 |  | 107.1 | 5.355 |
| 51.06 g/L KHP Buffer pH 5.5 | HOOCC6H4COOK | 204.22 | 5 | 10.94 | 214.2 | 10.71 |
| R-(+)-Limonene (815.8 g/L) | C10H16 | 136.23 | 8.2 | 100.0 | 122.58 | 6.129 |
| Water |  |  |  |  |  | 27.806 |
| **Compound** | **Formula** | **Mw** | **Stock concentration (g/L)** | **Final Concentration (g/L)** | **Final Concentration (mM)** | **Amount (g)** |
| Glucose | C6H12O6 | 180.16 |  | 21.42 | 118.89 | 1.071 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Medium** | **Final concentration after inoculation (g/L)** | **Working concentration (g/L)** | **Final volume (mL)** | **Concentrated medium (g/L)** | **Concentrated medium (mL)** | **21.43 g/L Glucose, pH 5.5 (1.071X) (mL)** |
| 2% glucose, 0.49% D-Limonene, pH 5.5 | 4.92 | 5.27 | 15.0 | 100.0 | 0.790 | 14.210 |
| 2% glucose, 0.9% D-Limonene, pH 5.5 | 9.00 | 9.64 | 15.0 | 100.0 | 1.446 | 13.554 |
| 2% glucose, 1.5% D-Limonene, pH 5.5 | 15.00 | 16.07 | 15.0 | 100.0 | 2.410 | 12.590 |
| 2% glucose, 2.1% D-Limonene, pH 5.5 | 21.00 | 22.49 | 15.0 | 100.0 | 3.374 | 11.626 |
| 2% glucose, 2.5% D-Limonene, pH 5.5 | 25.08 | 26.86 | 15.0 | 100.0 | 4.029 | 10.971 |
| 2% glucose, 3% D-Limonene, pH 5.5 | 30.00 | 32.13 | 15.0 | 100.0 | 4.820 | 10.181 |
|  |  |  |  | **SUM:** | **16.87** | **62.95** |

**Procedure**

1. Mix all liquid solutions in Table 1 in a 50 mL Falcon tube to prepare a concentrated stock solution. Work in a LAF-bench to keep stock solutions sterile.
2. Bring the mixture to the chemical room and add the solid compounds in Table 1 and adjust the pH with either NaOH or Tartaric acid.
3. Bring the solution back to the LAF-bench, complete the volume to 50 mL. **DO NOT** filter sterilize into a new tube as the terpene will be retained by the membrane of the filter.
4. Prepare the number of tubes indicated in Table 2 by labelling with corresponding concentration. Transfer the indicated volume into each tube. **TAKE CARE** to agitate the stock solution vigorously before the transfer.
5. Complete the volume to 15 mL by adding buffered minimal media with glucose.
6. Each solution of 15 mL is enough for 42 wells using 280 µL per well in a 96-well plate.

**MEDIA WITH DIFFERENT INHIBITORS, pH 3**

**21.43 g/L glucose + 10 g/L Acetic acid (1.071X), pH 3**

**Final volume (mL): 50**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Solution** | **Formula** | **Mw** | **Stock concentration (X)** | **Final Concentration (g/L)** | **Final Concentration (mL/L)** | **Amount (mL)** |
| 10X Salts + Vitamins + Trace (pH 3) |  |  | 10 |  | 107.1 | 5.355 |
| 5X TartarAc Buffer pH 3 | HO2CCH(OH)CH(OH)CO2H | 150.09 | 5 | 8.04 | 214.2 | 10.71 |
| Acetic acid (1049 g/L) | CH3CO2H | 60.05 | 104.90 | 10.00 | 9.53 | 0.477 |
| Water |  |  |  |  |  | 33.458 |
| **Compound** | **Formula** | **Mw** | **Stock concentration (g/L)** | **Final Concentration (g/L)** | **Final Concentration (mM)** | **Amount (g)** |
| Glucose | C6H12O6 | 180.16 |  | 21.42 | 118.89 | 1.071 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Medium** | **Final concentration after inoculation (g/L)** | **Working concentration (g/L)** | **Final volume (mL)** | **Concentrated medium (g/L)** | **Concentrated medium (mL)** | **21.43 g/L Glucose, pH 3 (1.071X) (mL)** |
| 2% glucose, 0.13% Acetic acid, pH 3 | 1.32 | 1.41 | 15.0 | 10.00 | 2.121 | 12.879 |
| 2% glucose, 0.20% Acetic acid, pH 3 | 2.00 | 2.14 | 15.0 | 10.00 | 3.213 | 11.787 |
| 2% glucose, 0.30% Acetic acid, pH 3 | 3.00 | 3.21 | 15.0 | 10.00 | 4.820 | 10.181 |
| 2% glucose, 0.40% Acetic acid, pH 3 | 4.00 | 4.28 | 15.0 | 10.00 | 6.426 | 8.574 |
| 2% glucose, 0.47% Acetic acid, pH 3 | 4.68 | 5.01 | 15.0 | 10.00 | 7.518 | 7.482 |
|  |  |  |  | **SUM:** | **24.10** | **50.90** |

**Procedure**

1. Mix all liquid solutions in Table 1 in a 50 mL Falcon tube to prepare a concentrated stock solution. Work in a LAF-bench to keep stock solutions sterile.
2. Bring the mixture to the chemical room and add the solid compounds in Table 1 and adjust the pH with either NaOH or Tartaric acid.
3. Bring the solution back to the LAF-bench, complete the volume to 50 mL and filter sterilize into a new tube.
4. Prepare the number of tubes indicated in Table 2 by labelling with corresponding concentration. Transfer the indicated volume into each tube.
5. Complete the volume to 15 mL by adding buffered minimal media with glucose.
6. Each solution of 15 mL is enough for 42 wells using 280 µL per well in a 96-well plate.

**21.43 g/L glucose + 0.75 g/L Ferulic acid (LVL5, 1.071X), pH 3**

**Final volume (mL): 50**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Solution** | **Formula** | **Mw** | **Stock concentration (X)** | **Final Concentration (g/L)** | **Final Concentration (mL/L)** | **Amount (mL)** |
| 10X Salts + Vitamins + Trace (pH 3) |  |  | 10 |  | 107.1 | 5.355 |
| 37.5 g/L TartarAc Buffer pH 3 | HO2CCH(OH)CH(OH)CO2H | 150.09 | 5 | 8.04 | 214.2 | 10.71 |
| Water |  |  |  |  |  | 33.935 |
| **Compound** | **Formula** | **Mw** | **Stock concentration (g/L)** | **Final Concentration (g/L)** | **Final Concentration (mM)** | **Amount (g)** |
| Glucose | C6H12O6 | 180.16 |  | 21.42 | 118.89 | 1.071 |
| Ferulic acid | C10H10O4 | 194.18 |  | 0.75 | 3.86 | 0.038 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Medium** | **Final concentration after inoculation (g/L)** | **Working concentration (g/L)** | **Final volume (mL)** | **Concentrated medium (g/L)** | **Concentrated medium (mL)** | **21.43 g/L Glucose, pH 3 (1.071X) (mL)** |
| 2% glucose, 0.006% Ferulic acid, pH 3 | 0.06 | 0.06 | 15.0 | 0.75 | 1.295 | 13.705 |
| 2% glucose, 0.019% Ferulic acid, pH 3 | 0.19 | 0.20 | 15.0 | 0.75 | 4.070 | 10.930 |
| 2% glucose, 0.038% Ferulic acid, pH 3 | 0.38 | 0.41 | 15.0 | 0.75 | 8.140 | 6.860 |
| 2% glucose, 0.057% Ferulic acid, pH 3 | 0.57 | 0.61 | 15.0 | 0.75 | 12.209 | 2.791 |
| 2% glucose, 0.070% Ferulic acid, pH 3 | 0.70 | 0.75 | 15.0 | 0.75 | 15.000 | 0.000 |
|  |  |  |  | **SUM:** | **40.71** | **34.29** |

**Procedure**

1. Mix all liquid solutions in Table 1 in a 50 mL Falcon tube to prepare a concentrated stock solution. Work in a LAF-bench to keep stock solutions sterile.
2. Bring the mixture to the chemical room and add the solid compounds in Table 1 and adjust the pH with either NaOH or Tartaric acid.
3. Bring the solution back to the LAF-bench, complete the volume to 50 mL and filter sterilize into a new tube.
4. Prepare the number of tubes indicated in Table 2 by labelling with corresponding concentration. Transfer the indicated volume into each tube.
5. Complete the volume to 15 mL by adding buffered minimal media with glucose.
6. Each solution of 15 mL is enough for 42 wells using 280 µL per well in a 96-well plate.

**21.43 g/L glucose + 50.1 g/L Succinic acid (1.071X), pH 3**

**Final volume (mL): 50**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Solution** | **Formula** | **Mw** | **Stock concentration (X)** | **Final Concentration (g/L)** | **Final Concentration (mL/L)** | **Amount (mL)** |
| 10X Salts + Vitamins + Trace (pH 3) |  |  | 10 |  | 107.1 | 5.355 |
| 37.5 g/L TartarAc Buffer pH 3 | HO2CCH(OH)CH(OH)CO2H | 150.09 | 5 | 8.04 | 214.2 | 10.71 |
| Water |  |  |  |  |  | 33.935 |
| **Compound** | **Formula** | **Mw** | **Stock concentration (g/L)** | **Final Concentration (g/L)** | **Final Concentration (mM)** | **Amount (g)** |
| Glucose | C6H12O6 | 180.16 |  | 21.42 | 118.89 | 1.071 |
| Succinic acid | C4H6O4 | 118.09 |  | 50.10 | 424.25 | 2.505 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Medium** | **Final concentration after inoculation (g/L)** | **Working concentration (g/L)** | **Final volume (mL)** | **Concentrated medium (g/L)** | **Concentrated medium (mL)** | **21.43 g/L Glucose, pH 3 (1.071X) (mL)** |
| 2% glucose, 1.3% Succinic acid, pH 3 | 13.20 | 14.14 | 15.0 | 50.10 | 4.233 | 10.767 |
| 2% glucose, 2%% Succinic acid, pH 3 | 20.00 | 21.42 | 15.0 | 50.10 | 6.413 | 8.587 |
| 2% glucose,3% Succinic acid, pH 3 | 30.00 | 32.13 | 15.0 | 50.10 | 9.620 | 5.380 |
| 2% glucose, 4% Succinic acid, pH 3 | 40.00 | 42.84 | 15.0 | 50.10 | 12.826 | 2.174 |
| 2% glucose, 4.7% Ferulic acid, pH 3 | 46.78 | 50.10 | 15.0 | 50.10 | 15.000 | 0.000 |
|  |  |  |  | **SUM:** | **48.09** | **26.91** |

**Procedure**

1. Mix all liquid solutions in Table 1 in a 50 mL Falcon tube to prepare a concentrated stock solution. Work in a LAF-bench to keep stock solutions sterile.
2. Bring the mixture to the chemical room and add the solid compounds in Table 1 and adjust the pH with either NaOH or Tartaric acid.
3. Bring the solution back to the LAF-bench, complete the volume to 50 mL and filter sterilize into a new tube.
4. Prepare the number of tubes indicated in Table 2 by labelling with corresponding concentration. Transfer the indicated volume into each tube.
5. Complete the volume to 15 mL by adding buffered minimal media with glucose.
6. Each solution of 15 mL is enough for 42 wells using 280 µL per well in a 96-well plate.

**21.43 g/L glucose + 100 g/L Pyruvic acid (1.071X), pH 3**

**Final volume (mL): 50**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Solution** | **Formula** | **Mw** | **Stock concentration (X)** | **Final Concentration (g/L)** | **Final Concentration (mL/L)** | **Amount (mL)** |
| 10X Salts + Vitamins + Trace (pH 3) |  |  | 10 |  | 107.1 | 5.355 |
| 37.5 g/L TartarAc Buffer pH 3 | HO2CCH(OH)CH(OH)CO2H | 150.09 | 5 | 8.04 | 214.2 | 10.71 |
| Pyruvic acid (1242 g/L) | C3H4O3 | 88.06 | 12.4 | 100.00 | 80.52 | 4.026 |
| Water |  |  |  |  |  | 29.909 |
| **Compound** | **Formula** | **Mw** | **Stock concentration (g/L)** | **Final Concentration (g/L)** | **Final Concentration (mM)** | **Amount (g)** |
| Glucose | C6H12O6 | 180.16 |  | 21.42 | 118.89 | 1.071 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Medium** | **Final concentration after inoculation (g/L)** | **Working concentration (g/L)** | **Final volume (mL)** | **Concentrated medium (g/L)** | **Concentrated medium (mL)** | **21.43 g/L Glucose, pH 3 (1.071X) (mL)** |
| 2% glucose, 1.3% Pyruvic acid, pH 3 | 13.20 | 14.14 | 15.0 | 100.00 | 2.121 | 12.879 |
| 2% glucose, 2% Pyruvic acid, pH 3 | 20.00 | 21.42 | 15.0 | 100.00 | 3.213 | 11.787 |
| 2% glucose, 3% Pyruvic acid, pH 3 | 30.00 | 32.13 | 15.0 | 100.00 | 4.820 | 10.181 |
| 2% glucose, 4% Pyruvic acid, pH 3 | 40.00 | 42.84 | 15.0 | 100.00 | 6.426 | 8.574 |
| 2% glucose, 4.6% Pyruvic acid, pH 3 | 46.80 | 50.12 | 15.0 | 100.00 | 7.518 | 7.482 |
|  |  |  |  | **SUM:** | **24.10** | **50.90** |

**Procedure**

1. Mix all liquid solutions in Table 1 in a 50 mL Falcon tube to prepare a concentrated stock solution. Work in a LAF-bench to keep stock solutions sterile.
2. Bring the mixture to the chemical room and add the solid compounds in Table 1 and adjust the pH with either NaOH or Tartaric acid.
3. Bring the solution back to the LAF-bench, complete the volume to 50 mL and filter sterilize into a new tube.
4. Prepare the number of tubes indicated in Table 2 by labelling with corresponding concentration. Transfer the indicated volume into each tube.
5. Complete the volume to 15 mL by adding buffered minimal media with glucose.
6. Each solution of 15 mL is enough for 42 wells using 280 µL per well in a 96-well plate.

**21.43 g/L glucose + 2 g/L Formic acid (1.071X), pH 3**

**Final volume (mL): 50**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Solution** | **Formula** | **Mw** | **Stock concentration (X)** | **Final Concentration (g/L)** | **Final Concentration (mL/L)** | **Amount (mL)** |
| 10X Salts + Vitamins + Trace (pH 3) |  |  | 10 |  | 107.1 | 5.355 |
| 5X TartarAc Buffer pH 3 | HO2CCH(OH)CH(OH)CO2H | 150.09 | 5 | 8.04 | 214.2 | 10.71 |
| Formic acid (1220 g/L) | CH2O2 | 46.03 | 610.0 | 2.00 | 1.64 | 0.082 |
| Water |  |  |  |  |  | 33.853 |
| **Compound** | **Formula** | **Mw** | **Stock concentration (g/L)** | **Final Concentration (g/L)** | **Final Concentration (mM)** | **Amount (g)** |
| Glucose | C6H12O6 | 180.16 |  | 21.42 | 118.89 | 1.071 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Medium** | **Final concentration after inoculation (g/L)** | **Working concentration (g/L)** | **Final volume (mL)** | **Concentrated medium (g/L)** | **Concentrated medium (mL)** | **21.43 g/L Glucose, pH 3 (1.071X) (mL)** |
| 2% glucose, 0.016% Formic acid, pH 3 | 0.16 | 0.17 | 15.0 | 2.00 | 1.285 | 13.715 |
| 2% glucose, 0.030% Formic acid, pH 3 | 0.30 | 0.32 | 15.0 | 2.00 | 2.410 | 12.590 |
| 2% glucose, 0.050% Formic acid, pH 3 | 0.50 | 0.54 | 15.0 | 2.00 | 4.016 | 10.984 |
| 2% glucose, 0.070% Formic acid, pH 3 | 0.70 | 0.75 | 15.0 | 2.00 | 5.623 | 9.377 |
| 2% glucose, 0.084% Formic acid, pH 3 | 0.84 | 0.90 | 15.0 | 2.00 | 6.747 | 8.253 |
|  |  |  |  | **SUM:** | **20.08** | **54.92** |

**Procedure**

1. Mix all liquid solutions in Table 1 in a 50 mL Falcon tube to prepare a concentrated stock solution. Work in a LAF-bench to keep stock solutions sterile.
2. Bring the mixture to the chemical room and add the solid compounds in Table 1 and adjust the pH with either NaOH or Tartaric acid.
3. Bring the solution back to the LAF-bench, complete the volume to 50 mL and filter sterilize into a new tube.
4. Prepare the number of tubes indicated in Table 2 by labelling with corresponding concentration. Transfer the indicated volume into each tube.
5. Complete the volume to 15 mL by adding buffered minimal media with glucose.
6. Each solution of 15 mL is enough for 42 wells using 280 µL per well in a 96-well plate.

**21.43 g/L glucose + 10 g/L Furfural (1.071X), pH 3**

**Final volume (mL): 50**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Solution** | **Formula** | **Mw** | **Stock concentration (X)** | **Final Concentration (g/L)** | **Final Concentration (mL/L)** | **Amount (mL)** |
| 10X Salts + Vitamins + Trace (pH 3) |  |  | 10 |  | 107.1 | 5.355 |
| 5X TartarAc Buffer pH 3 | HO2CCH(OH)CH(OH)CO2H | 150.09 | 5 | 8.04 | 214.2 | 10.71 |
| Furfural (1160 g/L) | C5H4O2 | 96.08 | 116.0 | 10.00 | 8.62 | 0.431 |
| Water |  |  |  |  |  | 33.504 |
| **Compound** | **Formula** | **Mw** | **Stock concentration (g/L)** | **Final Concentration (g/L)** | **Final Concentration (mM)** | **Amount (g)** |
| Glucose | C6H12O6 | 180.16 |  | 21.42 | 118.89 | 1.071 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Medium** | **Final concentration after inoculation (g/L)** | **Working concentration (g/L)** | **Final volume (mL)** | **Concentrated medium (g/L)** | **Concentrated medium (mL)** | **21.43 g/L Glucose, pH 3 (1.071X) (mL)** |
| 2% glucose, 0.04% Furfural, pH 3 | 0.40 | 0.43 | 15.0 | 10.00 | 0.639 | 14.361 |
| 2% glucose, 0.0825% Furfural, pH 3 | 0.825 | 0.88 | 15.0 | 10.00 | 1.325 | 13.675 |
| 2% glucose, 0.125% Furfural, pH 3 | 1.25 | 1.34 | 15.0 | 10.00 | 2.008 | 12.992 |
| 2% glucose, 0.25% Furfural, pH 3 | 2.50 | 2.68 | 15.0 | 10.00 | 4.016 | 10.984 |
| 2% glucose, 0.375% Furfural, pH 3 | 3.75 | 4.02 | 15.0 | 10.00 | 6.024 | 8.976 |
| 2% glucose, 0.46% Furfural, pH 3 | 4.60 | 4.93 | 15.0 | 10.00 | 7.390 | 7.610 |
|  |  |  |  | **SUM:** | **21.40** | **68.60** |

**Procedure**

1. Mix all liquid solutions in Table 1 in a 50 mL Falcon tube to prepare a concentrated stock solution. Work in a LAF-bench to keep stock solutions sterile.
2. Bring the mixture to the chemical room and add the solid compounds in Table 1 and adjust the pH with either NaOH or Tartaric acid.
3. Bring the solution back to the LAF-bench, complete the volume to 50 mL and filter sterilize into a new tube.
4. Prepare the number of tubes indicated in Table 2 by labelling with corresponding concentration. Transfer the indicated volume into each tube.
5. Complete the volume to 15 mL by adding buffered minimal media with glucose.
6. Each solution of 15 mL is enough for 42 wells using 280 µL per well in a 96-well plate.

**21.43 g/L glucose + 5.91 g/L Fumaric acid (1.071X), pH 3**

**Final volume (mL): 50**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Solution** | **Formula** | **Mw** | **Stock concentration (X)** | **Final Concentration (g/L)** | **Final Concentration (mL/L)** | **Amount (mL)** |
| 10X Salts + Vitamins + Trace (pH 3) |  |  | 10 |  | 107.1 | 5.355 |
| 5X TartarAc Buffer pH 3 | HO2CCH(OH)CH(OH)CO2H | 150.09 | 5 | 8.04 | 214.2 | 10.71 |
| Water |  |  |  |  |  | 33.935 |
| **Compound** | **Formula** | **Mw** | **Stock concentration (g/L)** | **Final Concentration (g/L)** | **Final Concentration (mM)** | **Amount (g)** |
| Glucose | C6H12O6 | 180.16 |  | 21.42 | 118.89 | 1.071 |
| Fumaric acid | C4H4O4 | 116.07 |  | 5.91 | 50.92 | 0.296 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Medium** | **Final concentration after inoculation (g/L)** | **Working concentration (g/L)** | **Final volume (mL)** | **Concentrated medium (g/L)** | **Concentrated medium (mL)** | **21.43 g/L Glucose, pH 3 (1.071X) (mL)** |
| 2% glucose, 0.048% Fumaric acid, pH 3 | 0.48 | 0.51 | 15.0 | 5.91 | 1.297 | 13.703 |
| 2% glucose, 0.15% Fumaric acid, pH 3 | 1.50 | 1.61 | 15.0 | 5.91 | 4.077 | 10.923 |
| 2% glucose, 0.3% Fumaric acid, pH 3 | 3.00 | 3.21 | 15.0 | 5.91 | 8.155 | 6.845 |
| 2% glucose, 0.45% Fumaric acid, pH 3 | 4.50 | 4.82 | 15.0 | 5.91 | 12.232 | 2.768 |
| 2% glucose, 0.59% Fumaric acid, pH 3 | 5.52 | 5.91 | 15.0 | 5.91 | 15.000 | 0.000 |
|  |  |  |  | **SUM:** | **40.76** | **34.24** |

**Procedure**

1. Mix all liquid solutions in Table 1 in a 50 mL Falcon tube to prepare a concentrated stock solution. Work in a LAF-bench to keep stock solutions sterile.
2. Bring the mixture to the chemical room and add the solid compounds in Table 1 and adjust the pH with either NaOH or Tartaric acid.
3. Bring the solution back to the LAF-bench, complete the volume to 50 mL and filter sterilize into a new tube.
4. Prepare the number of tubes indicated in Table 2 by labelling with corresponding concentration. Transfer the indicated volume into each tube.
5. Complete the volume to 15 mL by adding buffered minimal media with glucose.
6. Each solution of 15 mL is enough for 42 wells using 280 µL per well in a 96-well plate.