|  |  |  |  |
| --- | --- | --- | --- |
| **Amino acids** | **Stock solution [mM]** |  | **100 x stock [ml/l]** |
| Alanine | 1000 |  | 15 |
| Arginine | 500 |  | 20 |
| Cysteine | 250 |  | 4 |
| Glutamic acid | 500 |  | 20 |
| Glycine | 1000 |  | 15 |
| Histidin | 500 |  | 20 |
| Leucin | 500 |  | 60 |
| Lysin | 500 |  | 30 |
| Ornithin-HCl | 500 |  | 20 |
| Phenylalanine | 500 |  | 30 |
| Proline | 250 |  | 60 |
| Serine | 500 |  | 24 |
| Threonine | 500 |  | 40 |
| Tryptophane | 500 |  | 4 |
| Valin | 1000 |  | 10 |
| **total** |  |  | 362 |
|  |  |  | add 638 ml H2O |
|  |  |  |  |

All components of stock solutions were solved in sterile ddH2O and filter-sterilized. Stock solutions were stored at 4°C.

Composition of 100 x amino acid stock solution

Composition of 100 x organic acid stock solution

|  |  |  |  |
| --- | --- | --- | --- |
| **Organic acids** | **Stock solution [mM]** |  | **100 x stock [ml/l]** |
| Citrate | 100 |  | 20 |
| Fumarate | 500 |  | 1 |
| Malate | 100 |  | 10 |
| Pyruvate | 500 |  | 20 |
| Succinate | 250 |  | 80 |
| **total** |  |  | 131 |
|  |  |  | add 869 ml H2O |

Composition of 10 x salt / buffer mix

|  |  |  |  |
| --- | --- | --- | --- |
| **Salt/Buffer mix** | **Stock solution [mM]** |  | **10 x stock [ml/l]** |
| Na-P-buffer pH 7,2 | 1000 |  | 50 |
| K-P-buffer pH 7,2 | 1000 |  | 50 |
| NaCl | 5000 |  | 250 |
| KCl | 1000 |  | 200 |
| Urea | 5000 |  | 10 |
| MgSO4 | 1000 |  | 5 |
| Vitamin solution | 1000 x |  | 10 |
| Trace elements | 1000 x |  | 10 |
| **total** |  |  | 585 |
|  |  |  | add 415 ml H2O |
|  |  |  |  |

Composition of 1000 x vitamin and trace element stock solutions

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Vitamin solution** | **1000 x stock [g/l]** | | **Trace element solution** | | **1000 x stock [mg/l]** |
| Cyanocobalamin | 0.1 | | Zinkchloride | | 70 |
| p-Aminobenzoate | 0.08 | | Manganese dichloride x 4 H2O | | 100 |
| Biotin | 0.02 | | Boric acid | | 6 |
| Nicotinate | 0.2 | | Cobaltdichloride x 6 H2O | | 190 |
| Ca-D-Panthothenate | 0.1 | | Copper dichloride x 2 H2O | | 2 |
| Pyridoxamine-2HCl | 0.3 | | Nickel dichloride x 6 H2O | | 24 |
| Thiamindichloride | 0.2 |  | Di-sodium molybdate x 2 H2O | | 36 |
| Riboflavin | 0.2 |  |  |  |  |

To prepare SNM3 (Aminoacids and organic acids are three times concentrated compared to the concentration within nasal secretions that’s where the numbers below come from!)

30ml - 100x Aminosäure stock

+30ml - 100x organic acid

+100ml 10x salt buffer mix

+ 1ml 1000x vitamin & trace mix (to get 1x)

Fill to a final volume of 1L