**Medium to use**

**Unconstrained elements**

|  |  |  |
| --- | --- | --- |
| **Elements** | **Recon ID** | **ub** |
| **H2O** | h2o[e] | 1000 |
| **O2** | o2[e] | 1000 |
| **H** | h[e] | 1000 |
| **O2S** | o2s[e] | 1000 |
| **CO2** | Co2[e] | 1000 |
| **Pi** | pi[e] | 1000 |
| **H2O2** | h2o2[e] | 1000 |
| **HCO3** | hco3[e] | 1000 |
| **H2CO3** | h2co3[e] | 1000 |
| **CO** | co[e] | 1000 |

**Media**

- Plasmax medium

- Article: Vande Voorde et al. *Improving the metabolic fidelity of cancer models with a physiological cell culture medium*. Science Advances. 2019;5: eaau7314 (<10.1126/sciadv.aau7314>)

- Detailed information gathered from supplementary table S1 (<https://advances.sciencemag.org/content/advances/suppl/2018/12/21/5.1.eaau7314.DC1/aau7314_SM.pdf>)

- Medium: <https://ximbio.com/reagent/156371/plasmaxsuptmsup-cell-culture-medium-coming-soon#datasheet>

- HPLM

- Article: Cantor JR et al. *Physiologic Medium Rewires Cellular Metabolism and Reveals Uric Acid as an Endogenous Inhibitor of UMP Synthase*. Cell. 2017;169(2): 258-272.e17 ([doi.org/10.1016/j.cell.2017.03.023](https://doi.org/10.1016/j.cell.2017.03.023))

- Detailed information gathered from supplementary table S1 (<https://advances.sciencemag.org/content/advances/suppl/2018/12/21/5.1.eaau7314.DC1/aau7314_SM.pdf>)

- Medium:

- RPMI-1640:

- Article:

- Detailed information gathered from: : <https://ss-usa.s3.amazonaws.com/c/308472537/media/187615dfb9b2320e9776486215674958/Plasmax%28TM%29%20formulation%20compared%20to%20historic%20media.pdf>

- Medium:

Table summarising information on the media. Some components will not be used in the models, either because is part of the metabolism or because is not represented in Recon3D. Concentrations for each medium are in μM and were converted to mmol gDW-1 h-1 (*ubs* column). To do this, it was considered to have 1L of volume to 1gDW of cells and that the components could be fully consumed in 24 hours (as media should be changed every 24/48 hours in experiments). I.E: ub = metab\_media\_concentration (μM) \* 10-3 / 24 h.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Elements** | **ID** | **Plasmax** | **HPLM** | **RPMI-1640** |
| **Proteinogenic Amino Acids** | L-Alanine | ala\_L[e] | 510 | 430 |  |
| L-Arginine | arg\_L[e] | 64 | 110 | 1149 |
| L-Asparagine | asn\_L[e] | 41 | 50 | 379 |
| L-Aspartic acid | asp\_L[e] | 6 | 20 | 150 |
| L-Cysteine | cys\_L[e] | 33 | 40 |  |
| L-Glutamate | glu\_L[e] | 98 | 80 | 136 |
| L-Glutamine | gln\_L[e] | 650 | 550 | 2055 |
| Glycine | gly[e] | 330 | 300 | 133 |
| L-Histidine | his\_L[e] | 120 | 110 | 97 |
| L-Isoleucine | ile\_L[e] | 140 | 70 | 382 |
| L-Leucine | leu\_L[e] | 170 | 160 | 382 |
| L-Lysine | lys\_L[e] | 220 | 200 | 219 |
| L-Methionine | met\_L[e] | 30 | 30 | 101 |
| L-Phenylalanine | phe\_L[e] | 68 | 80 | 91 |
| L-Proline | pro\_L[e] | 360 | 200 | 174 |
| L-Serine | ser\_L[e] | 140 | 150 | 286 |
| L-Threonine | thr\_L[e] | 240 | 140 | 168 |
| L-Tryptophan | trp\_L[e] | 78 | 60 | 25 |
| L-Tyrosine | tyr\_L[e] | 74 | 80 | 111 |
| L-Valine | val\_L[e] | 230 | 220 | 171 |
| **Non-proteinogenic Amino Acids** | α-Aminobutyrate | C02356[e] | 41 | 20 |  |
| L-Citrulline | citr\_L[e] | 55 | 40 |  |
| L-Cystine | Lcystin[e] | 65 | 100 | 207.7 |
| L-Homocysteine | hcys\_L[e] | 9 |  |  |
| 4-Hydroxy-L-proline | 4hpro\_LT[e] | 13 | 20 | 152.7 |
| L-Ornithine | orn[e] | 80 | 10 |  |
| L-Pyroglutamate | 5oxpro[e] | 20 |  |  |
| **Amino Acids Derivatives** | L-Acetyl glycine | acgly[e] | 70 | 90 |  |
| L-Carnosine | carn[e] | 6 |  |  |
| Glutathione (reduced) | gthrd[e] | 37 | 25 | 3.3 |
| Taurine | taur[e] | 130 | 90 |  |
| N-Trimethylglycine (betaine) | glyb[e] | 72 | 70 |  |
| **Other Components** | Acetate | ac[e] | 42 | 40 |  |
| Acetone | acetone[e] | 55 | 60 |  |
| Acetyl carnitine | acrn[e] | 5 |  |  |
| Citrate | cit[e] | 114 | 130 |  |
| Carnitine | crn[e] | 46 | 40 |  |
| Creatine | creat[e] | 37 | 40 |  |
| Creatinine | crtn[e] | 74 | 75 |  |
| Formate | for[e] | 33 | 50 |  |
| Fructose | fru[e] |  | 40 |  |
| Galactose | gal[e] |  | 60 |  |
| D-Glucose | glc\_D[e] | 5560 | 5000 | 11111 |
| Glycerol | glyc[e] | 82 | 120 |  |
| 2-Hydroxybutyrate | 2hb[e] | 31 | 50 |  |
| 3-Hydroxybutyrate | bhb[e] | 77 | 50 |  |
| 3-Hydroxyisobutyrate | 3hmp[e] | 20 |  |  |
| Hypoxanthine | hxan[e] | 5 | 10 |  |
| Lactate | lac\_L[e]; lac\_D[e] | 500 | 1600 |  |
| Malonate | HC00319[e] |  | 10 |  |
| Methyl acetoacetate | - | 41 |  |  |
| Phenol Red | - | 25 | 14 | 13.3 |
| Pyruvate | pyr[e] | 100 | 50 |  |
| Succinate | succ[e] | 23 | 20 |  |
| Uracil | ura[e] | 2 |  |  |
| Urate | urate[e] | 270 | 350 |  |
| Urea | urea[e] | 3000 | 5000 |  |
| Uridine | uri[e] | 3 |  |  |
| **Inorganic Salts** | Ammonium Chloride | nh4[e] + cl[e] | 50 | 40 |  |
| Calcium Chloride | ca2[e] + 2 cl[e] | 1800 | 2350 |  |
| Calcium Nitrate | ca2[e] + 2 CE5643[e] |  | 40 | 424 |
| Magnesium Chloride | mg2[e] + 2 cl[e] |  | 480 |  |
| Magnesium Sulfate | mg2[e] + so4[e] | 813 | 350 | 407 |
| Potassium Chloride | k[e] + cl[e] | 5330 | 4100 | 5333 |
| Sodium Bicarbonate | na1[e] + hco3[e] | 26191 | 24000 | 23810 |
| Sodium Chloride | na1[e] + cl[e] | 118706 | 105000 | 103448 |
| Sodium Phosphate | 2 na1[e] + pi[e] | 1010 | 870 | 5634 |
| **Trace Elements** | Ammonium Metavanadate | nh4[e] + - | 0.0026 |  |  |
| Cupric Sulfate | cu2[e] + so4[e] | 0.0052 |  |  |
| Ferric Nitrate | fe3[e] + 3 CE5643[e] | 0.1238 |  |  |
| Ferric Sulfate | 2 fe3[e] + 3 so4[e] | 1.048 |  |  |
| Manganous Chloride | - + 2 cl[e] | 0.0002 |  |  |
| Sodium Selenite | 2 na1[e] + selni[e] | 0.0289 |  |  |
| Zinc Sulfate | zn2[e] + so4[e] | 1.5 |  |  |
| **Vitamins** | p-Aminobenzoate | - |  | 7.3 | 7.3 |
| Ascorbate | ascb\_L[e] | 62 |  |  |
| D-Biotin | btn[e] | 4.1 | 0.8 | 0.820 |
| Choline | chol[e] | 7.1 | 21.5 | 21.4 |
| Folate | fol[e] | 2.3 | 2.3 | 2.27 |
| Myo-Inositol | inost[e] | 11.1 | 194.3 | 194.4 |
| Niacinamide | ncam[e] | 8.2 | 8.2 | 8.2 |
| D-Pantothenic acid hemicalcium | 2 pnto\_R[e] and ca2[e] | 4.2 | 1.05 | 0.52 |
| Pyridoxine | Pydxn[e] | 4.9 | 4.9 | 4.9 |
| Riboflavin | ribflv[e] | 0.3 | 0.5 | 0.53 |
| Thiamine | thm[e] | 3 | 3 | 3 |
| Vitamin B12 | **aquacob(III)alamin** | 0.005 | 0.0037 | 0.0037 |

Final media table:

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Elements** | **Recon ID** | **Plasmax** | **HPLM** | **RPMI-1640** | **P\_ub** | **H\_ub** | **R\_ub** |
| L-Alanine | ala\_L[e] | 510 | 430 | 0 | 0,02125 | 0,01791667 | 0 |
| L-Arginine | arg\_L[e] | 64 | 110 | 1149 | 0,00266667 | 0,00458333 | 0,047875 |
| L-Asparagine | asn\_L[e] | 41 | 50 | 379 | 0,00170833 | 0,00208333 | 0,01579167 |
| L-Aspartic acid | asp\_L[e] | 6 | 20 | 150 | 0,00025 | 0,00083333 | 0,00625 |
| L-Cysteine | cys\_L[e] | 33 | 40 | 0 | 0,001375 | 0,00166667 | 0 |
| L-Glutamate | glu\_L[e] | 98 | 80 | 136 | 0,00408333 | 0,00333333 | 0,00566667 |
| L-Glutamine | gln\_L[e] | 650 | 550 | 2055 | 0,02708333 | 0,02291667 | 0,085625 |
| Glycine | gly[e] | 330 | 300 | 133 | 0,01375 | 0,0125 | 0,00554167 |
| L-Histidine | his\_L[e] | 120 | 110 | 97 | 0,005 | 0,00458333 | 0,00404167 |
| L-Isoleucine | ile\_L[e] | 140 | 70 | 382 | 0,00583333 | 0,00291667 | 0,01591667 |
| L-Leucine | leu\_L[e] | 170 | 160 | 382 | 0,00708333 | 0,00666667 | 0,01591667 |
| L-Lysine | lys\_L[e] | 220 | 200 | 219 | 0,00916667 | 0,00833333 | 0,009125 |
| L-Methionine | met\_L[e] | 30 | 30 | 101 | 0,00125 | 0,00125 | 0,00420833 |
| L-Phenylalanine | phe\_L[e] | 68 | 80 | 91 | 0,00283333 | 0,00333333 | 0,00379167 |
| L-Proline | pro\_L[e] | 360 | 200 | 174 | 0,015 | 0,00833333 | 0,00725 |
| L-Serine | ser\_L[e] | 140 | 150 | 286 | 0,00583333 | 0,00625 | 0,01191667 |
| L-Threonine | thr\_L[e] | 240 | 140 | 168 | 0,01 | 0,00583333 | 0,007 |
| L-Tryptophan | trp\_L[e] | 78 | 60 | 25 | 0,00325 | 0,0025 | 0,00104167 |
| L-Tyrosine | tyr\_L[e] | 74 | 80 | 111 | 0,00308333 | 0,00333333 | 0,004625 |
| L-Valine | val\_L[e] | 230 | 220 | 171 | 0,00958333 | 0,00916667 | 0,007125 |
| α-Aminobutyrate | C02356[e] | 41 | 20 | 0 | 0,00170833 | 0,00083333 | 0 |
| L-Citrulline | citr\_L[e] | 55 | 40 | 0 | 0,00229167 | 0,00166667 | 0 |
| L-Cystine | Lcystin[e] | 65 | 100 | 207.7 | 0,00270833 | 0,00416667 | 0,00865417 |
| L-Homocysteine | hcys\_L[e] | 9 | 0 | 0 | 0,000375 | 0 | 0 |
| 4-Hydroxy-L-proline | 4hpro\_LT[e] | 13 | 20 | 152.7 | 0,00054167 | 0,00083333 | 0,0063625 |
| L-Ornithine | orn[e] | 80 | 10 | 0 | 0,00333333 | 0,00041667 | 0 |
| L-Pyroglutamate | 5oxpro[e] | 20 | 0 | 0 | 0,00083333 | 0 | 0 |
| L-Acetyl glycine | acgly[e] | 70 | 90 | 0 | 0,00291667 | 0,00375 | 0 |
| L-Carnosine | carn[e] | 6 | 0 | 0 | 0,00025 | 0 | 0 |
| Glutathione (reduced) | gthrd[e] | 37 | 25 | 3.3 | 0,00154167 | 0,00104167 | 0,0001375 |
| Taurine | taur[e] | 130 | 90 | 0 | 0,00541667 | 0,00375 | 0 |
| N-Trimethylglycine (betaine) | glyb[e] | 72 | 70 | 0 | 0,003 | 0,00291667 | 0 |
| Acetate | ac[e] | 42 | 40 | 0 | 0,00175 | 0,00166667 | 0 |
| Acetone | acetone[e] | 55 | 60 | 0 | 0,00229167 | 0,0025 | 0 |
| Acetyl carnitine | acrn[e] | 5 |  | 0 | 0,00020833 | 0 | 0 |
| Citrate | cit[e] | 114 | 130 | 0 | 0,00475 | 0,00541667 | 0 |
| Carnitine | crn[e] | 46 | 40 | 0 | 0,00191667 | 0,00166667 | 0 |
| Creatine | creat[e] | 37 | 40 | 0 | 0,00154167 | 0,00166667 | 0 |
| Creatinine | crtn[e] | 74 | 75 | 0 | 0,00308333 | 0,003125 | 0 |
| Formate | for[e] | 33 | 50 | 0 | 0,001375 | 0,00208333 | 0 |
| Fructose | fru[e] | 0 | 40 | 0 | 0 | 0,00166667 | 0 |
| Galactose | gal[e] | 0 | 60 | 0 | 0 | 0,0025 | 0 |
| D-Glucose | glc\_D[e] | 5560 | 5000 | 11111 | 0,23166667 | 0,20833333 | 0,46295833 |
| Glycerol | glyc[e] | 82 | 120 | 0 | 0,00341667 | 0,005 | 0 |
| 2-Hydroxybutyrate | 2hb[e] | 31 | 50 | 0 | 0,00129167 | 0,00208333 | 0 |
| 3-Hydroxybutyrate | bhb[e] | 77 | 50 | 0 | 0,00320833 | 0,00208333 | 0 |
| 3-Hydroxyisobutyrate | 3hmp[e] | 20 | 0 | 0 | 0,00083333 | 0 | 0 |
| Hypoxanthine | hxan[e] | 5 | 10 | 0 | 0,00020833 | 0,00041667 | 0 |
| Lactate | lac\_L[e] | 500 | 1600 | 0 | 0,02083333 | 0,06666667 | 0 |
| Lactate | lac\_D[e] | 500 | 1600 | 0 | 0,02083333 | 0,06666667 | 0 |
| Malonate | HC00319[e] | 0 | 10 | 0 | 0 | 0,00041667 | 0 |
| Pyruvate | pyr[e] | 100 | 50 | 0 | 0,00416667 | 0,00208333 | 0 |
| Succinate | succ[e] | 23 | 20 | 0 | 0,00095833 | 0,00083333 | 0 |
| Uracil | ura[e] | 2 | 0 | 0 | 8,3333E-05 | 0 | 0 |
| Urate | urate[e] | 270 | 350 | 0 | 0,01125 | 0,01458333 | 0 |
| Urea | urea[e] | 3000 | 5000 | 0 | 0,125 | 0,20833333 | 0 |
| Uridine | uri[e] | 3 | 0 | 0 | 0,000125 | 0 | 0 |
| Ammonia | nh4[e] | 50,0026 | 40 | 0 | 0,00208344 | 0,00166667 | 0 |
| Chloride ion | cl[e] | 127686 | 114800 | 108781 | 5,32025 | 4,78333333 | 4,53254167 |
| Calcium | ca2[e] | 1804.2 | 2391.05 | 424.52 | 0,075175 | 0,09962708 | 0,01768833 |
| Nitrate | CE5643[e] | 0.3714 | 40 | 424 | 1,5475E-05 | 0,00166667 | 0,01766667 |
| Magnesium | mg2[e] | 813 | 830 | 407 | 0,033875 | 0,03458333 | 0,01695833 |
| Sulfate | so4[e] | 817.6492 | 350 | 407 | 0,03406872 | 0,01458333 | 0,01695833 |
| Potassium | k[e] | 5330 | 4100 | 5333 | 0,22208333 | 0,17083333 | 0,22220833 |
| Sodium | na1[e] | 146917.058 | 130740 | 138526 | 6,12154408 | 5,4475 | 5,77191667 |
| Hidrogen carbonate | hco3[e] | 26191 | 24000 | 23810 | 1,09129167 | 1 | 0,99208333 |
| Phosphate | pi[e] | 1010 | 870 | 5634 | 0,04208333 | 0,03625 | 0,23475 |
| Copper | cu2[e] | 0.0052 | 0 | 0 | 2,1667E-07 | 0 | 0 |
| Iron3 | fe3[e] | 2.2198 | 0 | 0 | 9,2492E-05 | 0 | 0 |
| ? Selenite | Only in cytoplasm | 0.0289 | 0 | 0 | 1,2042E-06 | 0 | 0 |
| Zinc | zn2[e] | 1.5 | 0 | 0 | 0,0000625 | 0 | 0 |
| D-Pantothenic acid | pnto\_R[e] | 8.4 | 2.1 | 1.04 | 0,00035 | 0,0000875 | 4,3333E-05 |
| Ascorbate | ascb\_L[e] | 62 | 0 | 0 | 0,00258333 | 0 | 0 |
| D-Biotin | btn[e] | 4.1 | 0.8 | 0.820 | 0,00017083 | 3,3333E-05 | 3,4167E-05 |
| Choline | chol[e] | 7.1 | 21.5 | 21.4 | 0,00029583 | 0,00089583 | 0,00089167 |
| Folate | fol[e] | 2.3 | 2.3 | 2.27 | 9,5833E-05 | 9,5833E-05 | 9,4583E-05 |
| Myo-Inositol | inost[e] | 11.1 | 194.3 | 194.4 | 0,0004625 | 0,00809583 | 0,0081 |
| Niacinamide | ncam[e] | 8.2 | 8.2 | 8.2 | 0,00034167 | 0,00034167 | 0,00034167 |
| Pyridoxine | pydxn[e] | 4.9 | 4.9 | 4.9 | 0,00020417 | 0,00020417 | 0,00020417 |
| Riboflavin | ribflv[e] | 0.3 | 0.5 | 0.53 | 0,0000125 | 2,0833E-05 | 2,2083E-05 |
| Thiamine | thm[e] | 3 | 3 | 3 | 0,000125 | 0,000125 | 0,000125 |
| Vitamin B12 | **aquacob(III)alamin** | 0.005 | 0.0037 | 0.0037 | 2,0833E-07 | 1,5417E-07 | 1,5417E-07 |

Source for the concentration of RPMI-1640: <https://ss-usa.s3.amazonaws.com/c/308472537/media/187615dfb9b2320e9776486215674958/Plasmax%28TM%29%20formulation%20compared%20to%20historic%20media.pdf>