**Medium to use**

Two types of media were considered when performed the reconstructions, those based on cell culture media, and those based on the average concentration of the exchange metabolites from the HumanGEM model in the human blood.

**Unconstrained elements**

This elements were considered to be freely accessible to the cells, **in all media**.

|  |  |  |
| --- | --- | --- |
| **Elements** | **Recon ID** | **ub** |
| **H2O** | h2o[e] | 1000 |
| **O2** | o2[e] | 1000 |
| **H** | h[e] | 1000 |

**Cell Cluture Media Used**

- 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](https://ximbio.com/reagent/156371/plasmaxsuptmsup-cell-culture-medium-coming-soon" \l "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](https://ss-usa.s3.amazonaws.com/c/308472537/media/187615dfb9b2320e9776486215674958/Plasmax(TM) formulation compared to historic media.pdf)

- Medium:

**Note:** Lipoic acid was added with the concentration from the *Serum Metabolome Database*, which is integrated in the *Human Metabolome Database* (<https://serummetabolome.ca/>), as it showed to be a metabolite without which HumanGEM could not produce biomass and it is not present in the formulations of the media used.

Tables summarising information on the media used. Some components will not be used in the models, either because they are not part of the metabolism (e.g. Phenol Red) or because they are not represented in HumanGEM (e.g. p-Aminobenzoate). Concentrations for each medium are in M.

Source table:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **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** | -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 | acetoacetate + activated methyl group | 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 used as base to calculate the respective exchange fluxes (in microM → passar os valores aqui para mM):

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Elements** | **Recon ID** | **Plasmax** | **HPLM** | **RPMI-1640** |
| L-Alanine | ala\_L[e] | 510 | 430 | 0 |
| 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 | 0 |
| 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 |
| -Aminobutyrate | C02356[e] | 41 | 20 | 0 |
| L-Citrulline | citr\_L[e] | 55 | 40 | 0 |
| L-Cystine | Lcystin[e] | 65 | 100 | 207.7 |
| L-Homocysteine | hcys\_L[e] | 9 | 0 | 0 |
| 4-Hydroxy-L-proline | 4hpro\_LT[e] | 13 | 20 | 152.7 |
| L-Ornithine | orn[e] | 80 | 10 | 0 |
| L-Pyroglutamate | 5oxpro[e] | 20 | 0 | 0 |
| L-Acetyl glycine | acgly[e] | 70 | 90 | 0 |
| L-Carnosine | carn[e] | 6 | 0 | 0 |
| Glutathione (reduced) | gthrd[e] | 37 | 25 | 3.3 |
| Taurine | taur[e] | 130 | 90 | 0 |
| N-Trimethylglycine (betaine) | glyb[e] | 72 | 70 | 0 |
| Acetate | ac[e] | 42 | 40 | 0 |
| Acetone | acetone[e] | 55 | 60 | 0 |
| Acetyl carnitine | acrn[e] | 5 | 0 | 0 |
| Citrate | cit[e] | 114 | 130 | 0 |
| Carnitine | crn[e] | 46 | 40 | 0 |
| Creatine | creat[e] | 37 | 40 | 0 |
| Creatinine | crtn[e] | 74 | 75 | 0 |
| Formate | for[e] | 33 | 50 | 0 |
| Fructose | fru[e] | 0 | 40 | 0 |
| Galactose | gal[e] | 0 | 60 | 0 |
| D-Glucose | glc\_D[e] | 5560 | 5000 | 11111 |
| Glycerol | glyc[e] | 82 | 120 | 0 |
| 2-Hydroxybutyrate | 2hb[e] | 31 | 50 | 0 |
| 3-Hydroxybutyrate | bhb[e] | 77 | 50 | 0 |
| 3-Hydroxyisobutyrate | 3hmp[e] | 20 | 0 | 0 |
| Hypoxanthine | hxan[e] | 5 | 10 | 0 |
| Lactate | lac\_L[e] | 500 | 1600 | 0 |
| Lactate | lac\_D[e] | 500 | 1600 | 0 |
| Malonate | HC00319[e] | 0 | 10 | 0 |
| Acetoacetate | acac[e] | 41 |  |  |
| ?Activated methyl group | Only in cytoplasm | 41 |  |  |
| Pyruvate | pyr[e] | 100 | 50 | 0 |
| Succinate | succ[e] | 23 | 20 | 0 |
| Uracil | ura[e] | 2 | 0 | 0 |
| Urate | urate[e] | 270 | 350 | 0 |
| Urea | urea[e] | 3000 | 5000 | 0 |
| Uridine | uri[e] | 3 | 0 | 0 |
| Ammonia | nh4[e] | 50,0026 | 40 | 0 |
| Chloride ion | cl[e] | 127686 | 114800 | 108781 |
| Calcium | ca2[e] | 1804.2 | 2391.05 | 424.52 |
| Nitrate | CE5643[e] | 0.3714 | 40 | 424 |
| Magnesium | mg2[e] | 813 | 830 | 407 |
| Sulfate | so4[e] | 817.6492 | 350 | 407 |
| Potassium | k[e] | 5330 | 4100 | 5333 |
| Sodium | na1[e] | 146917.058 | 130740 | 138526 |
| Hidrogen carbonate | hco3[e] | 26191 | 24000 | 23810 |
| Phosphate | pi[e] | 1010 | 870 | 5634 |
| Copper | cu2[e] | 0.0052 | 0 | 0 |
| Iron3 | fe3[e] | 2.2198 | 0 | 0 |
| ? Selenite | Only in cytoplasm | 0.0289 | 0 | 0 |
| Zinc | zn2[e] | 1.5 | 0 | 0 |
| D-Pantothenic acid | pnto\_R[e] | 8.4 | 2.1 | 1.04 |
| Ascorbate | ascb\_L[e] | 62 | 0 | 0 |
| 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 |
| 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 |
| Lipoic acid |  | 0.077 | 0.077 | 0.077 |

**Blood medium**

The metabolite concentrations for this medium were based on the average concentration that the exchange metabolites from the HumanGEM model have in the human blood. This information was taken from the *Serum Metabolome Database*, which is integrated in the *Human Metabolome Database* (<https://serummetabolome.ca/>).