**Supplemental Table 1a**  Direct Peptide Reactivity Assay (DPRA) Study 2 -- Lysine Depletion

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Peptide Peak Area at 220 nm | Percent Peptide Depletion | Mean Percent Peptide Depletion | SD of Percent Peptide Depletion | CV of Percent Peptide Depletion |
| \*1-Butyl-1-methylpyrrolidinium (Acetonitrile) | 2438971 | 0.7% | 0.2% | 0.4% | N/A |
| 2453448 | 0.1% |
| 2457422 | -0.1% |
| \*1-Butyl-3-methylimidazolium chloride (Acetonitrile) | 2436065 | 0.8% | 0.8% | 0.3% | 31.5% |
| 2443305 | 0.5% |
| 2430041 | 1.0% |
| 1-Ethyl-3-methylimidazolium chloride (Acetonitrile) | 2410026 | 1.9% | 0.6% | 1.2% | N/A |
| 2449108 | 0.3% |
| 2466114 | -0.4% |
| \* N-Butyl-pyridinium chloride (Acetonitrile) | 2443892 | -0.8% | *1-1.2%* | 0.8% | N/A |
| 2476418 | -2.1% |
| 2445404 | -0.8% |

\*Lysine peptide peak splitting was observed, but peaks could not be integrated separately. Total peptide peak area was integrated.

1Negative values are reported as “0” when calculating mean depletion for the DPRA Summary Results.

**Supplemental Table 1b**  Direct Peptide Reactivity Assay (DPRA) Study 2 -- Cysteine Depletion

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Peptide Peak Area at 220 nm | Percent Peptide Depletion | Mean Percent Peptide Depletion | SD of Percent Peptide Depletion | CV of Percent Peptide Depletion |
| 1-Butyl-1-methylpyrrolidinium (Acetonitrile) | 2357428 | -3.8% | *1-2.8%* | 1.7% | N/A |
| 2358809 | -3.8% |
| 2292252 | -0.9% |
| 1-Butyl-3-methylimidazolium chloride (Acetonitrile) | 2164456 | 4.7% | 6.9% | 2.4% | 35.3% |
| 2125686 | 6.4% |
| 2056864 | 9.5% |
| 1-Ethyl-3-methylimidazolium chloride (Acetonitrile) | 2344198 | -3.2% | *1-1.2%* | 2.2% | N/A |
| 2244380 | 1.2% |
| 2310487 | -1.7% |
| N-Butyl-pyridinium chloride (Acetonitrile) | 2240488 | -0.1% | 1-0.6% | 2.3% | N/A |
| 2310118 | -3.2% |
| 2206743 | 1.4% |

1Negative values are reported as “0” when calculating mean depletion for the DPRA Summary Results.

**Supplemental Table 2a**  Direct Peptide Reactivity Assay (DPRA) Study 2 – Control Data for BMIM, EMIM, and BMPY, Lysine Peptide

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Lysine Peptide** | Peptide Peak Area at 220 nm | Peptide Conc. (mM) | Mean Peptide Conc. (mM) | SD of Mean Peptide Conc. (mM) | CV of Peptide Conc. | Mean Peptide Conc. (mM) | SD of Mean Peptide Conc. (mM) | CV of Peptide Conc. |
| Reference Control A, Rep 1 | 2439253 | 0.496 | 0.504 | 0.007 | 1.5% |  |  |  |
| Reference Control A, Rep 2 | 2508543 | 0.510 |  |  |  |
| Reference Control A, Rep 3 | 2491089 | 0.507 |  |  |  |
| Reference Control B, Rep 1 | 2580291 | 0.525 | 0.512 | 0.012 | 2.3% | 0.503 | 0.00994 | 2.0% |
| Reference Control B, Rep 2 | 2498720 | 0.508 |
| Reference Control B, Rep 3 | 2474607 | 0.503 |
| Reference Control B, Rep 4 | 2445751 | 0.497 | 0.497 | 0.001 | 0.1% |
| Reference Control B, Rep 5 | 2442406 | 0.497 |
| Reference Control B, Rep 6 | 2441311 | 0.496 |
| Reference Control C, Rep 1 (acetonitrile) | 2494421 | 0.507 | 0.499 | 0.008 | 1.5% |
| Reference Control C, Rep 2 (acetonitrile) | 2450274 | 0.498 |
| Reference Control C, Rep 3 (acetonitrile) | 2422041 | 0.492 |
|  |  |  |  |  |  |  |  |  |
|  | Peptide Peak Area at 220 nm | Percent Peptide Depletion | Mean Percent Peptide Depletion | SD of Percent Peptide Depletion | CV of Percent Peptide Depletion |  |  |  |
| †Cinnamic aldehyde | 1009159 | 58.9% | 54.0% | 4.6% | 8.5% |  |  |  |
| 1148754 | 53.2% |  |  |  |
| 1232898 | 49.8% |  |  |  |

†A co-eluting peak was observed but is < 10% of the mean peptide peak area from Reference Control C, acetonitrile and is therefore considered baseline noise.

**Supplemental Table 2b**  Direct Peptide Reactivity Assay (DPRA) Study 2 – Control Data for BMIM, EMIM, and BMPY, Cysteine Peptide

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Cysteine Peptide** | Peptide Peak Area at 220 nm | Peptide Conc. (mM) | Mean Peptide Conc. (mM) | SD of Mean Peptide Conc. (mM) | CV of Peptide Conc. | Mean Peptide Conc. (mM) | SD of Mean Peptide Conc. (mM) | CV of Peptide Conc. |
| Reference Control A, Rep 1 | 2453504 | 0.512 | 0.513 | 0.002 | 0.3% |  |  |  |
| Reference Control A, Rep 2 | 2452395 | 0.512 |  |  |  |
| Reference Control A, Rep 3 | 2465777 | 0.515 |  |  |  |
| Reference Control B, Rep 1 | 2278606 | 0.475 | 0.479 | 0.012 | 2.5% | 0.467 | 0.02172 | 4.7% |
| Reference Control B, Rep 2 | 2245803 | 0.469 |
| Reference Control B, Rep 3 | 2359517 | 0.492 |
| Reference Control B, Rep 4 | 2177352 | 0.454 | 0.448 | 0.027 | 6.0% |
| Reference Control B, Rep 5 | 2255837 | 0.471 |
| Reference Control B, Rep 6 | 2006374 | 0.418 |
| Reference Control C, Rep 1 (acetonitrile) | 2343066 | 0.489 | 0.474 | 0.013 | 2.8% |
| Reference Control C, Rep 2 (acetonitrile) | 2224042 | 0.464 |
| Reference Control C, Rep 3 (acetonitrile) | 2247603 | 0.469 |
|  |  |  |  |  |  |  |  |  |
|  | Peptide Peak Area at 220 nm | Percent Peptide Depletion | Mean Percent Peptide Depletion | SD of Percent Peptide Depletion | CV of Percent Peptide Depletion |  |  |  |
| Cinnamic aldehyde | 610948 | 73.1% | 72.3% | 0.7% | 1.0% |  |  |  |
| 640828 | 71.8% |  |  |  |
| 639389 | 71.9% |  |  |  |

**Supplemental Table 3a**  Direct Peptide Reactivity Assay (DPRA) Study 2 – Control Data for NBuPY, Lysine Peptide

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Lysine Peptide** | Peptide Peak Area at 220 nm | Peptide Conc. (mM) | Mean Peptide Conc. (mM) | SD of Mean Peptide Conc. (mM) | CV of Peptide Conc. | Mean Peptide Conc. (mM) | SD of Mean Peptide Conc. (mM) | CV of Peptide Conc. |
| Reference Control A, Rep 1 | 2508397 | 0.513 | 0.506 | 0.012 | 2.3% |  |  |  |
| Reference Control A, Rep 2 | 2406393 | 0.492 |  |  |  |
| Reference Control A, Rep 3 | 2503719 | 0.512 |  |  |  |
| Reference Control B, Rep 1 | 2441104 | 0.499 | 0.498 | 0.009 | 1.8% | 0.496 | 0.00727 | 1.5% |
| Reference Control B, Rep 2 | 2392671 | 0.489 |
| Reference Control B, Rep 3 | 2477380 | 0.507 |
| Reference Control B, Rep 4 | 2470249 | 0.505 | 0.494 | 0.009 | 1.9% |
| Reference Control B, Rep 5 | 2390519 | 0.489 |
| Reference Control B, Rep 6 | 2389413 | 0.489 |
| Reference Control C, Rep 1 (acetonitrile) | 2402062 | 0.491 | 0.496 | 0.006 | 1.1% |
| Reference Control C, Rep 2 (acetonitrile) | 2417549 | 0.494 |
| Reference Control C, Rep 3 (acetonitrile) | 2455735 | 0.502 |
|  |  |  |  |  |  |
|  | Peptide Peak Area at 220 nm | Percent Peptide Depletion | Mean Percent Peptide Depletion | SD of Percent Peptide Depletion | CV of Percent Peptide Depletion |
| †Cinnamic aldehyde | 1091850 | 55.0% | 52.7% | 2.3% | 4.3% |
| 1150109 | 52.6% |
| 1199498 | 50.5% |

†A co-eluting peak was observed but is < 10% of the mean peptide peak area from Reference Control C, acetonitrile and is therefore considered baseline noise.

**Supplemental Table 3b** Direct Peptide Reactivity Assay (DPRA) Study 2 – Control Data for NBuPY, Cysteine Peptide

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Cysteine Peptide** | Peptide Peak Area at 220 nm | Peptide Conc. (mM) | Mean Peptide Conc. (mM) | SD of Mean Peptide Conc. (mM) | CV of Peptide Conc. | Mean Peptide Conc. (mM) | SD of Mean Peptide Conc. (mM) | CV of Peptide Conc. |
| Reference Control A, Rep 1 | 2291599 | 0.527 | 0.514 | 0.023 | 4.4% |  |  |  |
| Reference Control A, Rep 2 | 2298576 | 0.528 |  |  |  |
| Reference Control A, Rep 3 | 2127041 | 0.488 |  |  |  |
| Reference Control B, Rep 1 | 2387397 | 0.549 | 0.540 | 0.013 | 2.4% | 0.525 | 0.02144 | 4.1% |
| Reference Control B, Rep 2 | 2373782 | 0.546 |
| Reference Control B, Rep 3 | 2283770 | 0.525 |
| Reference Control B, Rep 4 | 2241066 | 0.515 | 0.521 | 0.010 | 1.9% |
| Reference Control B, Rep 5 | 2320872 | 0.533 |
| Reference Control B, Rep 6 | 2246834 | 0.516 |
| Reference Control C, Rep 1 (acetonitrile) | 2315170 | 0.532 | 0.514 | 0.032 | 6.2% |
| Reference Control C, Rep 2 (acetonitrile) | 2320878 | 0.533 |
| Reference Control C, Rep 3 (acetonitrile) | 2077638 | 0.477 |
|  |  |  |  |  |  |  |  |  |
|  | Peptide Peak Area at 220 nm | Percent Peptide Depl. | Mean Percent Peptide Depl. | SD of Percent Peptide Depl. | CV of Percent Peptide Depl. |  |  |  |
| Cinnamic aldehyde | 612695 | 72.6% | 72.8% | 0.6% | 0.8% |  |  |  |
| 593168 | 73.5% |  |  |  |
| 617999 | 72.4% |  |  |  |

**Supplemental Table 4a** KeratinoSens™ Assay Study 2 – Gene Induction Summary for BMPY and BMIM

| **Chemical** | ***Concentration (µM)*** | | | | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ***0.98*** | | ***1.95*** | | ***3.91*** | ***7.81*** | ***15.63*** | ***31.25*** | ***62.50*** | ***125.00*** | ***250.00*** | ***500.00*** | ***1000.00*** | ***2000.00*** |
| ***1-Butyl-1-methylpyrrolidinium chloride*** | |  | | | |  |  |  |  |  |  |  |  |  |
| Run 1 – Induction | 0.65 | | 0.98 | | 1.02 | 0.95 | 1.05 | 1.11 | 1.02 | 1.18 | 1.29 | 1.61 | 1.87 | 2.31 |
| *Run 1 – RLU 1* | *206* | | *8364* | | *9227* | *8772* | *9921* | *10820* | *9803* | *9945* | *12794* | *12943* | *15559* | *19313* |
| *Run 1 – RLU 2* | *10019* | | *9808* | | *10171* | *8880* | *10874* | *9459* | *9430* | *9587* | *11645* | *16788* | *17816* | *24115* |
| *Run 1 – RLU 3* | *8644* | | *9248* | | *9056* | *8894* | *8600* | *10358* | *9209* | *13094* | *11183* | *15053* | *18305* | *20508* |
| *Run 1 – p-value* | *0.384* | | *0.782* | | *0.757* | *0.309* | *0.560* | *0.184* | *0.714* | *0.256* | *0.014* | *0.009* | *0.001* | *0.001* |
| Run 2 – Induction | 1.06 | | 1.09 | | 1.16 | 1.17 | 1.18 | 1.12 | 1.15 | 1.24 | 1.42 | 1.63 | 2.10 | 4.73 |
| *Run 2 – RLU 1* | *6056* | | *6115* | | *7333* | *8025* | *6056* | *7277* | *7170* | *7775* | *8993* | *8742* | *13030* | *32372* |
| *Run 2 – RLU 2* | *8118* | | *8498* | | *8236* | *6935* | *9238* | *6999* | *7549* | *8285* | *8577* | *10211* | *12308* | *29827* |
| *Run 2 – RLU 3* | *6164* | | *6390* | | *6677* | *7455* | *7328* | *7114* | *7278* | *7671* | *9579* | *12018* | *14498* | *27410* |
| *Run 2 – p-value* | *0.6011* | | *0.4701* | | *0.0945* | *0.0332* | *0.2861* | *0.0075* | *0.0049* | *0.0026* | *0.0011* | *0.0147* | *0.0005* | *0.0001* |
| **Average Induction** | **0.86** | | **1.04** | | **1.09** | **1.06** | **1.12** | **1.11** | **1.09** | **1.21** | **1.36** | **1.62** | **1.99** | **3.52** |
| **SD** | **0.29** | | **0.08** | | **0.10** | **0.16** | **0.09** | **0.01** | **0.09** | **0.04** | **0.10** | **0.01** | **0.16** | **1.71** |
|  |  | |  | |  |  |  |  |  |  |  |  |  |  |
| ***1-Butyl-3-methylimidazolium chloride*** | | | |  | |  |  |  |  |  |  |  |  |  |
| Run 1 – Induction | 0.62 | | 0.90 | | 1.01 | 1.00 | 0.96 | 1.06 | 1.04 | 1.01 | 1.17 | 1.07 | 1.26 | 1.64 |
| *Run 1 – RLU 1* | *160* | | *8153* | | *9454* | *9214* | *8414* | *10049* | *8531* | *8930* | *10731* | *8929* | *11847* | *14533* |
| *Run 1 – RLU 2* | *9896* | | *8428* | | *9985* | *8999* | *10319* | *10132* | *10058* | *10206* | *11534* | *10991* | *12882* | *17288* |
| *Run 1 – RLU 3* | *7971* | | *8442* | | *8700* | *9469* | *8158* | *9253* | *10446* | *9042* | *10304* | *9857* | *10441* | *13677* |
| *Run 1 – p-value* | *0.333* | | *0.075* | | *0.905* | *0.851* | *0.682* | *0.360* | *0.630* | *0.892* | *0.045* | *0.438* | *0.041* | *0.007* |
| Run 2 – Induction | 1.05 | | 0.96 | | 1.04 | 1.08 | 1.18 | 1.09 | 1.18 | 1.16 | 1.29 | 1.30 | 1.47 | 3.93 |
| *Run 2 – RLU 1* | *7555* | | *6085* | | *6429* | *6439* | *7416* | *6503* | *7159* | *7233* | *7883* | *8136* | *9158* | *25842* |
| *Run 2 – RLU 2* | *6311* | | *6291* | | *7121* | *7858* | *7396* | *7704* | *7691* | *7441* | *7709* | *7927* | *8860* | *22470* |
| *Run 2 – RLU 3* | *6216* | | *5983* | | *6360* | *6489* | *7666* | *6679* | *7716* | *7500* | *9017* | *8654* | *9975* | *26109* |
| *Run 2 – p-value* | *0.5392* | | *0.1486* | | *0.4220* | *0.3279* | *0.0019* | *0.2225* | *0.0068* | *0.0025* | *0.0134* | *0.0018* | *0.0012* | *0.0001* |
| **Average Induction** | **0.84** | | **0.93** | | **1.02** | **1.04** | **1.07** | **1.07** | **1.11** | **1.08** | **1.23** | **1.18** | **1.37** | **2.79** |
| **SD** | **0.30** | | **0.04** | | **0.02** | **0.06** | **0.15** | **0.02** | **0.10** | **0.11** | **0.08** | **0.16** | **0.15** | **1.62** |
|  |  | |  | |  |  |  |  |  |  |  |  |  |  |

RLU – Relative Luminescence Units; p-value is calculated using a two-sample Student’s T-Test between the three technical replicates and the corresponding vehicle treatment technical replicates

(1-Butyl-1-methylpyrrolidinium chloride and 1-Butyl-3-methylimidazolium chloride Vehicle Run 1 – 8976.8, 10097.33, 8858 RLU; Vehicle Run 2 – 6380.5, 6612.833, 6186 RLU; Refer to the methods section for description of the induction calculations.

**Supplemental Table 4b** KeratinoSens™ Assay Study 2 – Gene Induction Summary for EMIM and NBuPY

| **Chemical** | ***Concentration (µM)*** | | | | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ***0.98*** | | ***1.95*** | | ***3.91*** | ***7.81*** | ***15.63*** | ***31.25*** | ***62.50*** | ***125.00*** | ***250.00*** | ***500.00*** | ***1000.00*** | ***2000.00*** |
| ***1-Ethyl-3-methylimidazolium chloride*** | |  | | | |  |  |  |  |  |  |  |  |  |
| Run 1 – Induction | 0.92 | | 0.99 | | 0.91 | 1.00 | 0.96 | 0.95 | 0.99 | 0.94 | 0.92 | 1.06 | 1.12 | 1.82 |
| *Run 1 – RLU 1* | *8037* | | *7563* | | *7317* | *7843* | *7072* | *6778* | *7818* | *7259* | *8047* | *8732* | *9027* | *13486* |
| *Run 1 – RLU 2* | *8575* | | *8747* | | *8393* | *8678* | *9376* | *8791* | *8271* | *8742* | *7144* | *9911* | *9680* | *16139* |
| *Run 1 – RLU 3* | *7709* | | *9788* | | *8404* | *9793* | *8805* | *9436* | *10004* | *8904* | *9150* | *9214* | *10697* | *17969* |
| *Run 1 – p-value* | *0.103* | | *0.864* | | *0.139* | *0.936* | *0.608* | *0.589* | *0.867* | *0.413* | *0.318* | *0.324* | *0.143* | *0.006* |
| Run 2 – Induction | 0.93 | | 0.98 | | 1.05 | 1.08 | 1.00 | 1.13 | 1.13 | 1.08 | 1.13 | 1.22 | 1.18 | 1.30 |
| *Run 2 – RLU 1* | *4366* | | *5280* | | *5249* | *5648* | *5614* | *6841* | *5614* | *5643* | *5839* | *6390* | *6588* | *8138* |
| *Run 2 – RLU 2* | *5039* | | *4739* | | *5869* | *5889* | *5363* | *5700* | *6321* | *5162* | *6561* | *6129* | *5859* | *6070* |
| *Run 2 – RLU 3* | *5115* | | *5186* | | *5167* | *5199* | *4597* | *4971* | *5531* | *5894* | *5130* | *6262* | *5727* | *5833* |
| *Run 2 – p-value* | *0.280* | | *0.637* | | *0.371* | *0.162* | *0.960* | *0.300* | *0.081* | *0.187* | *0.195* | *0.002* | *0.040* | *0.112* |
| **Average Induction** | **0.93** | | **0.99** | | **0.98** | **1.04** | **0.98** | **1.04** | **1.06** | **1.01** | **1.02** | **1.14** | **1.15** | **1.56** |
| **SD** | **0.01** | | **0.01** | | **0.10** | **0.06** | **0.03** | **0.13** | **0.10** | **0.10** | **0.15** | **0.11** | **0.04** | **0.37** |
|  |  | |  | |  |  |  |  |  |  |  |  |  |  |
| ***N-Butyl-pyridinium chloride*** | | | |  | |  |  |  |  |  |  |  |  |  |
| Run 1 – Induction | 1.01 | | 0.99 | | 1.01 | 1.14 | 1.13 | 1.23 | 1.29 | 1.48 | 1.52 | 1.76 | 2.08 | 4.00 |
| *Run 1 – RLU 1* | *5943* | | *4782* | | *5020* | *5250* | *5098* | *6704* | *6767* | *7721* | *7317* | *9034* | *11969* | *21389* |
| *Run 1 – RLU 2* | *5810* | | *6370* | | *6286* | *7612* | *7170* | *6945* | *8109* | *9017* | *9130* | *10505* | *10947* | *23526* |
| *Run 1 – RLU 3* | *5515* | | *6021* | | *6105* | *6851* | *7352* | *7450* | *7228* | *8555* | *9710* | *10505* | *12214* | *22602* |
| *Run 1 – p-value* | *0.9918* | | *0.9659* | | *0.9276* | *0.3555* | *0.3849* | *0.0391* | *0.0392* | *0.0070* | *0.0212* | *0.0022* | *0.0004* | *0.00002* |
| Run 2 – Induction | 0.86 | | 1.04 | | 0.94 | 0.99 | 0.94 | 1.15 | 0.98 | 1.29 | 1.39 | 1.55 | 2.07 | 2.34 |
| *Run 2 – RLU 1* | *4209* | | *5044* | | *4212* | *4651* | *4680* | *4646* | *2804* | *5288* | *7043* | *7003* | *10815* | *10525* |
| *Run 2 – RLU 2* | *4636* | | *6213* | | *6047* | *5898* | *4926* | *6965* | *6542* | *7770* | *8703* | *10608* | *10721* | *14204* |
| *Run 2 – RLU 3* | *4798* | | *5201* | | *4818* | *5235* | *5302* | *6763* | *6404* | *7377* | *6262* | *7031* | *10785* | *12214* |
| *Run 2 – p-value* | *0.0864* | | *0.6948* | | *0.6836* | *0.9495* | *0.3858* | *0.3524* | *0.9749* | *0.1371* | *0.0570* | *0.0762* | *0.00004* | *0.0031* |
| **Average Induction** | **0.94** | | **1.01** | | **0.98** | **1.07** | **1.04** | **1.19** | **1.13** | **1.38** | **1.46** | **1.66** | **2.07** | **3.17** |
| **SD** | **0.11** | | **0.03** | | **0.05** | **0.11** | **0.14** | **0.06** | **0.22** | **0.14** | **0.09** | **0.15** | **0.01** | **1.17** |
|  |  | |  | |  |  |  |  |  |  |  |  |  |  |

RLU – Relative Luminescence Units; p-value is calculated using a two-sample Student’s T-Test between the three technical replicates and the corresponding vehicle treatment technical replicates

1-Ethyl-3-methylimidazolium chloride Vehicle Run 1 – 9253.667, 8476.167, 8740.5 RLU; Vehicle Run 2 – 5194.833, 5373.667, 4953 RLU; N-Butyl-pyridinium chloride Vehicle Run 1 – 5053.167, 5932.5, 6269.667 RLU; Vehicle Run 2 – 4827.833, 5789.333, 5258.833 RLU). Refer to the methods section for description of the induction calculations.

**Supplemental Table 4c KeratinoSens™ Assay Study 2 – Cell Viability**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ***Viability Average (%)*** | | | | | | | | | | | |
| **Concentration (µM)** | ***0.98*** | ***1.95*** | ***3.91*** | ***7.81*** | ***15.63*** | ***31.25*** | ***62.50*** | ***125.00*** | ***250.00*** | ***500.00*** | ***1000.00*** | ***2000.00*** |
| **BMPY** | 95.05 | 104.35 | 105.07 | 100.83 | 103.98 | 132.12 | 90.64 | 95.42 | 96.29 | 104.10 | 114.98 | 129.62 |
| **BMIM** | 97.59 | 104.37 | 106.53 | 97.73 | 106.25 | 110.50 | 100.21 | 114.67 | 127.08 | 123.07 | 124.15 | 117.21 |
| **EMIM** | 135.15 | 110.70 | 110.84 | 106.76 | 90.48 | 96.92 | 106.91 | 93.69 | 96.35 | 95.75 | 101.17 | 98.95 |
| **NBUPY** | 109.20 | 100.89 | 87.79 | 108.30 | 108.35 | 106.90 | 120.75 | 119.05 | 131.87 | 161.61 | 163.49 | 128.74 |

1All compounds were tested at a top concentration of 2000 µM. Results shown are mean values from two independent experiments.

**Supplemental Table 5a KeratinoSens Control Data for 1-Butyl-1-methylpyrrolidinium chloride (BMPY) & 1-Butyl-3-methylimidazolium chloride (BMIM)**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Quality control: Induction values Reference** | | | | |  |  | **Criteria** |  | **Quality control: Variability blank** | | | |
| **Cinnamic aldehyde** | ***4.00*** | ***8.00*** | ***16.00*** | ***32.00*** | ***64.00*** | **EC 1.5** | **EC 1.5** | **Ind. 64 uM** | | **% standard deviation blanks** | | |
| rep1 | 1.27 | 1.49 | 1.75 | 2.61 | 10.57 | 8.18 | ***TRUE*** | ***FALSE*** |  | 9.1 | ***ACCEPTED*** | |
| rep2 | 1.23 | 1.38 | 1.76 | 2.15 | 5.18 | 10.52 | ***TRUE*** | ***TRUE*** |  | 9.3 | ***ACCEPTED*** | |
| ***Average*** | ***1.25*** | ***1.44*** | ***1.76*** | ***2.38*** | ***7.88*** | ***9.35*** |  |  |  |  |  |  |
| ***StDEV*** | ***0.03*** | ***0.08*** | ***0.01*** | ***0.32*** | ***3.81*** |  |  |  |  |  |  |  |

Rep 2 results accepted due to cinnamic aldehyde meeting acceptance criteria for EC1.5 and dose-dependent gene induction.

**Supplemental Table 5b KeratinoSens Control Data for 1-Ethyl-3-methylimidazolium chloride (EMIM)**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Quality control: Induction values Reference** | | | | |  |  | **Criteria** |  | **Quality control: Variability blank** | | | |
| **Cinnamic aldehyde** | ***4.00*** | ***8.00*** | ***16.00*** | ***32.00*** | ***64.00*** | **EC 1.5** | **EC 1.5** | **Ind. 64 uM** | | **% standard deviation blanks** | | |
| rep1 | 1.17 | 1.23 | 1.58 | 2.57 | 6.45 | 14.22 | ***TRUE*** | ***TRUE*** |  | 10.0 | ***ACCEPTED*** | |
| rep2 | 1.17 | 1.26 | 1.46 | 2.07 | 3.68 | 16.94 | ***TRUE*** | ***TRUE*** |  | 14.1 | ***ACCEPTED*** | |
| **Average** | **1.17** | **1.25** | **1.52** | **2.32** | **5.06** | **15.58** |  |  |  |  |  |  |
| **StDEV** | ***0.00*** | ***0.02*** | ***0.08*** | ***0.36*** | ***1.96*** |  |  |  |  |  |  |  |

**Supplemental Table 5c KeratinoSens Control Data for N-Butyl-pyridinium chloride (NBuPY)**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Quality control: Induction values Reference** | | | | |  |  | **Criteria** |  | **Quality control: Variability blank** | | | |
| **Cinnamic aldehyde** | ***4.00*** | ***8.00*** | ***16.00*** | ***32.00*** | ***64.00*** | **EC 1.5** | **EC 1.5** | **Ind. 64 uM** | | **% standard deviation blanks** | | |
| rep1 | 1.27 | 1.43 | 1.60 | 2.34 | 4.55 | 11.28 | ***TRUE*** | ***TRUE*** |  | 14.8 | ***ACCEPTED*** | |
| rep2 | 1.09 | 1.34 | 1.45 | 2.06 | 3.25 | 17.26 | ***TRUE*** | ***TRUE*** |  | 11.4 | ***ACCEPTED*** | |
| **Average** | ***1.18*** | ***1.39*** | ***1.52*** | ***2.20*** | ***3.90*** | ***14.27*** |  |  |  |  |  |  |
| **StDEV** | ***0.12*** | ***0.06*** | ***0.10*** | ***0.20*** | ***0.92*** |  |  |  |  |  |  |  |

Rep = replicate, StDev = Standard deviation, EC=Effective Concentration

**Supplemental Table 6 h-CLAT Individual Run Results**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | **Run 1** | | | **Run 2** | | | **Run 3** | | |
| **Compound** | **Treatment** | **CD86 RFI** | **CD54 RFI** | **IgG1 Viability** | **CD86 RFI** | **CD54 RFI** | **IgG1 Viability** | **CD86 RFI** | **CD54 RFI** | **IgG1 Viability** |
| **BMPY** | 5000 µg/ml | 108.86 | 248.92 | 76.49 | 88.99 | 207.15 | 83.10 |
| 4167 µg/ml | 96.57 | 183.71 | 92.88 | 90.70 | 201.02 | 80.46 |
| 3472 µg/ml | 115.99 | 195.36 | 91.99 | 85.45 | 188.51 | 93.13 |
| 2894 µg/ml | 104.12 | 173.15 | 93.69 | 80.97 | 102.99 | 95.83 |
| 2411 µg/ml | 109.97 | 144.71 | 95.33 | 76.60 | 159.70 | 95.07 |
| 2009 µg/ml | 108.67 | 136.12 | 95.98 | 89.86 | 118.85 | 96.02 |
| 1674 µg/ml | 107.90 | 128.87 | 96.90 | 91.52 | 118.55 | 96.74 |
| 1395 µg/ml | 96.88 | 123.89 | 96.74 | 76.50 | 103.69 | 96.79 |
| **BMIM** | 5000 µg/ml | 114.68 | 299.98 | 34.45 | 76.58 | 188.10 | 13.71 |
| 4167 µg/ml | 107.45 | 324.39 | 56.96 | 84.11 | 212.20 | 23.73 |
| 3472 µg/ml | 111.08 | 302.32 | 60.05 | 72.65 | 272.05 | 64.55 |
| 2894 µg/ml | 108.36 | 317.70 | 73.86 | 71.79 | 270.14 | 71.61 |
| 2411 µg/ml | 91.72 | 299.86 | 85.37 | 74.57 | 266.43 | 84.77 |
| 2009 µg/ml | 104.75 | 246.66 | 91.37 | 78.42 | 265.37 | 90.71 |
| 1674 µg/ml | 99.70 | 217.99 | 94.98 | 89.75 | 228.50 | 93.52 |
| 1395 µg/ml | 100.71 | 223.57 | 94.02 | 82.12 | 419.52 | 93.46 |
| **EMIM** | 5000 µg/ml | 108.77 | 208.97 | 58.75 | 85.52 | 136.60 | 85.09 | 100.49 | 162.31 | 74.04 |
| 4167 µg/ml | 88.82 | 174.87 | 76.34 | 83.10 | 140.67 | 90.20 | 92.90 | 130.35 | 87.41 |
| 3472 µg/ml | 86.61 | 154.67 | 87.21 | 82.63 | 112.06 | 95.67 | 95.37 | 125.52 | 91.59 |
| 2894 µg/ml | 92.12 | 146.39 | 92.15 | 67.75 | 124.70 | 95.70 | 90.29 | 112.06 | 94.51 |
| 2411 µg/ml | 81.34 | 126.84 | 94.69 | 73.65 | 103.51 | 95.98 | 96.50 | 102.57 | 95.49 |
| 2009 µg/ml | 80.84 | 124.54 | 95.05 | 71.91 | 115.64 | 96.01 | 97.84 | 98.60 | 95.47 |
| 1674 µg/ml | 85.16 | 120.26 | 95.78 | 75.42 | 104.11 | 96.30 | 90.37 | 93.45 | 96.39 |
| 1395 µg/ml | 95.33 | 116.40 | 96.74 | 74.06 | 98.14 | 96.37 | 85.16 | 84.14 | 96.41 |
| **NBuPY** | 5000 µg/ml | 98.39 | 234.90 | 36.06 | 77.07 | 170.52 | 28.48 |
| 4167 µg/ml | 91.74 | 221.82 | 78.27 | 57.05 | 170.52 | 53.32 |
| 3472 µg/ml | 93.45 | 238.46 | 70.48 | 63.40 | 210.49 | 67.26 |
| 2894 µg/ml | 87.08 | 232.68 | 84.55 | 68.67 | 193.80 | 82.34 |
| 2411 µg/ml | 89.88 | 212.19 | 89.71 | 63.95 | 209.52 | 87.77 |
| 2009 µg/ml | 74.51 | 189.61 | 93.00 | 63.51 | 195.23 | 91.64 |
| 1674 µg/ml | 87.94 | 184.69 | 93.16 | 65.41 | 340.56 | 92.83 |
| 1395 µg/ml | 88.86 | 196.56 | 94.81 | 66.88 | 161.65 | 92.99 |

**h-CLAT Control Data**

**Supplemental Table 7a**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Run 1 – BMPY, BMIM, EMIM, NBuPY** | | | | | | | | | |
| **Compound** | **Treatment** | **CD86** | **CD54** | **IgG1** | **CD86 RFI** | **CD54 RFI** | **IgG1 Viability** | **CD86 Ratio** | **CD54 Ratio** |
| Controls | Media | 3921.92 | 3834.37 | 2635.72 |  |  | 97.65 | 148.80 | 145.48 |
| 0.2% DMSO | 3874.31 | 3774.98 | 2513.41 | 105.81 | 105.25 | 97.67 | 154.15 | 150.19 |
| 4.0 µg/ml DNCB | 12519.55 | 8674.71 | 3075.73 | ***693.94*** | ***443.81*** | 73.41 |  |  |

**Supplemental Table 7b**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Run 2 – BMPY, BMIM, EMIM, NBuPY** | | | | | | | | | |
| **Compound** | **Treatment** | **CD86** | **CD54** | **IgG1** | **CD86 RFI** | **CD54 RFI** | **IgG1 Viability** | **CD86 Ratio** | **CD54 Ratio** |
| Controls | Media | 4001.64 | 3783.18 | 2631.72 |  |  | 97.32 | 152.05 | 143.75 |
| 0.2% DMSO | 3969.62 | 3541.86 | 2447.83 | 111.09 | 95.01 | 96.93 | 162.17 | 144.69 |
| 4.0 µg/ml DNCB | 12402.32 | 9169.60 | 3178.45 | ***606.12*** | ***547.62*** | 70.34 |  |  |

**Supplemental Table 7c**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Run 3 – EMIM** | | | | | | | | | | |
| **Compound** | **Treatment** | **CD86** | **CD54** | **IgG1** | **CD86 RFI** | **CD54 RFI** | **IgG1 Viability** | **CD86 Ratio** | **CD54 Ratio** |
| Controls | Media | 5165.41 | 4991.93 | 2804.26 |  |  | 96.25 | 184.20 | 178.01 |
| 0.2% DMSO | 5122.93 | 4740.29 | 2790.39 | 98.79 | 89.13 | 96.72 | 183.59 | 169.88 |
| 4.0 µg/ml DNCB | 15403.25 | 14246.90 | 2987.74 | ***532.27*** | ***577.42*** | 67.18 |  |  |