**Table S1.** Complete list of the screened mutants with respective ‘BLANK’-wells (no enzyme) and ‘wt’-wells (containing wild-type enzyme).

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| well type | **Plate 1** | **Eapp[a]** | well type | **Plate 2** | **Eapp[a]** | well type | **Plate 3** | **Eapp[a]** | well type | **Plate 4** | **Eapp[a]** |
| **mutants**  **library** | A01 | 0.84 | **mutants**  **library** | A01 | 1.07 | **mutants**  **library** | A01 | 1.13[d] | **mutants**  **library** | A01 | 0.98 |
| A02 | 4.36[b] | A02 | 1.02 | A02 | 1.12 | A02 | 1.07 |
| A03 | 0.82 | A03 | 1.65 | A03 | 2.44 | A03 | 3.55 |
| A04 | 0.94[c] | A04 | 1.01 | A04 | 1.53 | A04 | 1.08 |
| A05 | 0.85 | A05 | 1.83 | A05 | 1.45 | A05 | 1.16 |
| A06 | 0.87 | A06 | 0.97 | A06 | 2.67 | A06 | 1.02 |
| A07 | 0.83 | A07 | 1.07 | A07 | 2.15 | A07 | 1.08 |
| A08 | 0.90 | A08 | 1.04 | A08 | 1.08 | A08 | 1.00 |
| A09 | 0.86 | A09 | 3.88 | A09 | 1.45 | A09 | 0.94 |
| A10 | 0.83 | A10 | 1.02 | A10 | 1.03 | A10 | 1.02 |
| A11 | 0.81 | A11 | 0.91 | A11 | 2.62 | A11 | 1.09 |
| A12 | 0.90 | A12 | 1.09 | A12 | 1.01 | A12 | 0.85 |
| B01 | 3.08 | B01 | 1.05 | B01 | 1.25 | B01 | 5.33 |
| B03 | 0.78[e] | B03 | 0.98 | B03 | 1.14 | B03 | 1.11 |
| B04 | 0.83 | B04 | 3.46 | B04 | 1.08 | B04 | 5.04 |
| B05 | 0.85 | B05 | 0.96 | B05 | 1.19 | B05 | 4.53 |
| B06 | 0.80 | B06 | 0.94 | B06 | 2.63 | B06 | 1.08 |
| B07 | 0.87 | B07 | 0.97 | B07 | 1.16 | B07 | 1.07 |
| B08 | 0.84 | B08 | 0.97 | B08 | 1.11 | B08 | 0.96 |
| B09 | 0.80 | B09 | 1.03 | B09 | 1.15 | B09 | 0.94 |
| B10 | 0.84 | B10 | 0.90 | B10 | 2.70 | B10 | 1.31 |
| B12 | 0.90 | B12 | 1.07 | B12 | 1.12 | B12 | 1.14 |
| C01 | 6.03 | C01 | 0.98 | C01 | 1.19 | C01 | 0.97 |
| C02 | 0.82 | C02 | 0.96 | C02 | 1.16 | C02 | 1.00 |
| C03 | 0.78 | C03 | 1.23 | C03 | 1.37 | C03 | 1.12 |
| C04 | 4.56 | C04 | 0.99 | C04 | 1.06 | C04 | 1.13 |
| C05 | 0.82 | C05 | 0.95 | C05 | 1.16 | C05 | 0.99 |
| C06 | 0.83 | C06 | 1.00 | C06 | 1.13 | C06 | 1.58 |
| C07 | 0.80 | C07 | 2.62 | C07 | 1.12 | C07 | 1.09 |
| C08 | 0.82 | C08 | 0.99 | C08 | 1.49 | C08 | 0.99 |
| C09 | 2.93 | C09 | 3.87 | C09 | 1.05 | C09 | 0.87 |
| C10 | 2.32 | C10 | 1.00 | C10 | 1.02 | C10 | 1.01 |
| C11 | 0.73 | C11 | 1.02 | C11 | 1.08 | C11 | 2.87 |
| C12 | 0.94 | C12 | 1.04 | C12 | 1.15 | C12 | 2.18 |
| D01 | 0.85 | D01 | 0.97 | D01 | 1.09 | D01 | 2.63 |
| D02 | 5.69 | D02 | 0.97 | D02 | 1.12 | D02 | 2.53 |
| D03 | 0.79 | D03 | 4.78 | D03 | 1.09 | D03 | 2.69 |
| D04 | 0.83 | D04 | 1.06 | D04 | 2.85 | D04 | 2.89 |
| D05 | 0.90 | D05 | 3.99 | D05 | 1.12 | D05 | 2.71 |
| D06 | 0.79 | D06 | 0.99 | D06 | 1.30 | D06 | 2.72 |
| D07 | 0.80 | D07 | 0.99 | D07 | 1.04 | D07 | 3.10 |
| D08 | 0.84 | D08 | 1.03 | D08 | 1.12 | D08 | 3.12 |
| D09 | 1.12 | D09 | 1.19 | D09 | 2.94 | D09 | 2.71 |
| D10 | 0.82 | D10 | 1.01 | D10 | 1.07 | D10 | 2.79 |
| D11 | 0.86 | D11 | 0.91 | D11 | 1.08 | D11 | 2.85 |
| D12 | 0.84 | D12 | 1.05 | D12 | 1.10 | D12 | 2.50 |
| E01 | 0.84 | E01 | 1.02 | E01 | 2.24 | E01 | 2.47 |
| E02 | 0.83 | E02 | 0.93 | E02 | 1.13 | E02 | 2.78 |
| E03 | 0.80 | E03 | 1.19 | E03 | 2.87 | E03 | 2.73 |
| E04 | 0.83 | E04 | 0.97 | E04 | 1.01 | E04 | 2.58 |
| E05 | 0.91 | E05 | 2.03 | E05 | 1.12 | E05 | 2.69 |
| E06 | 0.81 | E06 | 1.02 | E06 | 2.53 | E06 | 2.53 |
| E07 | 0.83 | E07 | 1.00 | E07 | 1.11 | E07 | 2.74 |
| E08 | 4.63 | E08 | 0.96 | E08 | 1.20 | E08 | 3.02 |
| E09 | 0.79 | E09 | 1.49 | E09 | 1.11 | E09 | 1.68 |
| E10 | 4.68 | E10 | 1.01 | E10 | 1.11 | E10 | 3.13 |
| E11 | 0.78 | E11 | 0.93 | E11 | 2.37 | E11 | 2.92 |
| E12 | 0.83 | E12 | 1.03 | E12 | 1.07 | E12 | 2.74 |
| F01 | 0.84 | F01 | 1.09 | F01 | 1.11 | F01 | 2.94 |
| F02 | 0.82 | F02 | 1.07 | F02 | 1.11 | F02 | 3.06 |
| F03 | 0.80 | F03 | 0.99 | F03 | 1.08 | F03 | 2.51 |
| F04 | 0.83 | F04 | 2.76 | F04 | 1.08 | F04 | 2.65 |
| F05 | 0.89 | F05 | 1.02 | F05 | 2.20 | F05 | 2.68 |
| F06 | 0.78 | F06 | 2.82 | F06 | 1.12 | F06 | 3.00 |
| F07 | 1.06 | F07 | 2.87 | F07 | 1.12 | F07 | 2.70 |
| F08 | 0.78 | F08 | 1.01 | F08 | 1.07 | F08 | 1.06 |
| F09 | 0.98 | F09 | 1.07 | F09 | 1.39 | F09 | 1.11 |
| F10 | 0.77 | F10 | 0.96 | F10 | 0.81 | F10 | 2.67 |
| F11 | 0.82 | F11 | 0.96 | F11 | 1.02 | F11 | 1.10 |
| F12 | 5.34 | F12 | 1.17 | F12 | 2.95 | F12 | 2.71 |
| G01 | 0.82 | G01 | 0.93 | G01 | 1.13 | G01 | 1.03 |
| G03 | 4.46 | G02 | 1.00 | G02 | 1.12 | G02 | 1.06 |
| G04 | 0.86 | G04 | 0.99 | G03 | 2.06 | G03 | 1.98 |
| G05 | 0.87 | G05 | 1.28 | G05 | 1.12 | G04 | 1.05 |
| G06 | 0.79 | G06 | 0.95 | G06 | 1.06 | G06 | 2.56 |
| G07 | 0.81 | G07 | 0.96 | G07 | 1.10 | G07 | 2.32 |
| G08 | 0.84 | G08 | 0.96 | G08 | 1.06 | G09 | 1.06 |
| G09 | 0.84 | G09 | 1.00 | G10 | 3.27 | G10 | 1.03 |
| G10 | 0.77 | G11 | 2.05 | G11 | 1.17 | G11 | 1.56 |
| G12 | 0.87 | G12 | 1.01 | G12 | 1.10 | G12 | 2.54 |
| **BLANK** | B02 | 0.83 | **BLANK** | B02 | 1.36 | **BLANK** | B02 | 1.18 | **BLANK** | B02 | 1.18 |
| B11 | 0.84 | B11 | 0.82 | B11 | 1.24 | B11 | 0.96 |
| G02 | 0.80 | G03 | 1.04 | G04 | 1.14 | G05 | 1.02 |
| G11 | 0.79 | G10 | 1.01 | G09 | 1.52 | G08 | 1.20 |
| **wt** | H01 | 2.55 | **wt** | H01 | 2.50 | **wt** | H01 | 2.35 | **wt** | H01 | 2.58 |
| H02 | 2.55 | H02 | 2.66 | H02 | 2.58 | H02 | 2.61 |
| H03 | 2.56 | H03 | 2.36 | H03 | 2.50 | H03 | 2.64 |
| H04 | 2.62 | H04 | 2.31 | H04 | 2.56 | H04 | 2.75 |
| H05 | 2.56 | H05 | 2.47 | H05 | 2.69 | H05 | 2.39 |
| H06 | 2.45 | H06 | 2.42 | H06 | 2.40 | H06 | 2.63 |
| H07 | 2.67 | H07 | 2.31 | H07 | 2.64 | H07 | 2.58 |
| H08 | 2.43 | H08 | 2.36 | H08 | 2.61 | H08 | 2.71 |
| H09 | 2.45 | H09 | 2.56 | H09 | 2.70 | H09 | 2.58 |
| H10 | 2.38 | H10 | 2.32 | H10 | 2.51 | H10 | 2.48 |
| H11 | 2.70 | H11 | 2.48 | H11 | 2.61 | H11 | 2.36 |
| H12 | 2.64 | H12 | 2.48 | H12 | 2.76 | H12 | 2.69 |
| [a] The Eapp was calculated as the ratio of activities; (ΔOD/min)(*R*)/(ΔOD/min)(*S*) [b] Indicates that EappHIT was higher than the highest Eappwt (out of 12 replicates per plate). [c] Indicates that the EappHIT was higher than the highest EappBLANK (out of 4 replicates per plate and equal to the compound’s auto-hydrolysis in aqueous solution) and lower than the lowest Eappwt (out of 12 replicates per plate). [d] Indicates that the EappHIT was lower than the lowest EappBLANK (out of 4 replicates per plate and equal to the compound’s auto-hydrolysis in aqueous solution). [e] Indicates that the EappHIT was lower than 1 and lower than the lowest EappBLANK (out of 4 replicates per plate and equal to the compound’s auto-hydrolysis in aqueous solution). | | | | | | | | | | | |