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| --- | --- | --- | --- | --- | --- |
| **Mutant** | **Protein yield** | **KM** | ***k*cat** | **KI** | ***k*cat/KM** |
|  | **mg/mL** | **mM** | **min-1** | **mM** | **M-1min-1** |
| BglB | 1.2 | 5.00 ± 0.2 | 880 ± 10 |  | 176,000 ± 8000 |
| S14A | 0.6 | 8.25 ± 1.02 | 320 ± 11 |  | 38,823 ± 4,972 |
| S16A | 0.83 | 14.01 ± 0.40 | 154 ± 1 |  | 10,997 ± 331 |
| S17E | 1.01 | 7.32 ± 0.38 | 641 ± 9 |  | 87,596 ± 4,719 |
| S17A | 0.65 | 18.45 ± 3.72 | 848 ± 76 |  | 45,978 ± 10,135 |
| Y18A | 0.17 | 31.55 ± 3.61 | 197 ± 9 |  | 6,230 ± 773 |
| Q19A | 0.26 |  |  |  | 11 ± 3 |
| Q19C | 0.4 |  |  |  | < 10 |
| Q19S | 0.43 |  |  |  | 13 ± 3 |
| W34A | ND |  |  |  |  |
| V52G | 0.97 | 8.25 ± 0.54 | 687 ± 13 |  | 83,371 ± 5,707 |
| F72A | 0.44 | 5.47 ± 0.28 | 613 ± 8 |  | 112,224 ± 6,000 |
| R76A | ND |  |  |  |  |
| I91E | 0.49 | 6.71 ± 0.79 | 846 ± 35 |  | 126,071 ± 15,714 |
| H101R | 1.03 | 10.62 ± 0.53 | 1059 ± 16 |  | 99,708 ± 5,225 |
| H119A | 1.21 | 15.10 ± 3.36 | 143 ± 11 |  | 9,483 ± 2,222 |
| H119E | ND |  |  |  |  |
| H119N | 1.02 | 23.22 ± 2.20 | 2 ± <0 |  | 82 ± 8 |
| W120A | 0.16 |  |  |  | < 10 |
| W120F | 0.78 | 16.08 ± 2.07 | 472 ± 21 |  | 29,334 ± 3,980 |
| W120H | 1 | 89.18 ± 4.31 | 84 ± 2 |  | 943 ± 53 |
| V147S | 0.23 | 6.45 ± 0.62 | 5 ± <1 |  | 706 ± 70 |
| E154D | 1.42 | 3.46 ± 0.76 | 878 ± 47 |  | 254,004 ± 57,175 |
| N163A | 0.74 | 11.95 ± 0.91 | 7 ± <1 |  | 558 ± 44 |
| N163C | 1.1 | 5.42 ± 0.32 | 26 ± <1 |  | 4,766 ± 291 |
| N163D | 1.05 | 15.19 ± 1.41 | 12 ± <1 |  | 789 ± 77 |
| E164A | 0.42 | 1.01 ± 0.17 | < 10 |  | 190 ± 33 |
| Y166P | 0.18 | 2.50 ± 0.45 | 27 ± 1 | 94.95 ± 10.18 | 10,596 ± 1,981 |
| C167A | 0.48 | 14.56 ± 1.27 | 479 ± 14 |  | 32,884 ± 3,026 |
| C167Q | 0.94 | 4.92 ± 0.19 | 504 ± 6 | 590.71 ± 86.56 | 102,415 ± 4,149 |
| L171A | 0.38 | 11.09 ± 0.42 | 807 ± 9 |  | 72,719 ± 2,851 |
| L171R | 1.06 | 3.36 ± 0.23 | 403 ± 7 |  | 120,146 ± 8,506 |
| T175R | 0.86 | 3.59 ± 0.15 | 801 ± 8 |  | 223,033 ± 9,663 |
| E177A | 0.96 | 5.98 ± 0.22 | 986 ± 10 |  | 164,804 ± 6,408 |
| E177K | 0.95 | 6.19 ± 0.30 | 555 ± 7 | 362.94 ± 36.97 | 89,609 ± 4,493 |
| E177L | 0.77 | 7.48 ± 0.36 | 670 ± 10 |  | 89,478 ± 4,555 |
| H178A | 0.25 | 7.67 ± 0.73 | 113 ± 3 | 173.34 ± 42.79 | 14,697 ± 1,463 |
| A192S | 1.17 | 5.09 ± 0.18 | 946 ± 10 |  | 185,848 ± 6,994 |
| T218A | 0.98 | 6.51 ± 0.94 | 464 ± 18 |  | 71,280 ± 10,669 |
| L219A | 0.47 | 7.87 ± 0.60 | 199 ± 5 |  | 25,262 ± 2,010 |
| N220A | 0.61 | 10.27 ± 0.68 | 405 ± 8 |  | 39,425 ± 2,745 |
| N220H | 1.12 | 5.14 ± 0.21 | 123 ± 1 |  | 23,874 ± 1,031 |
| M221A | 0.73 | 6.25 ± 0.60 | 547 ± 15 |  | 87,554 ± 8,701 |
| E222A | 0.29 | 0.63 ± 0.15 | 90 ± 4 | 95.24 ± 13.70 | 143,604 ± 36,130 |
| E222H | 0.7 | 8.54 ± 0.53 | 160 ± 3 |  | 18,695 ± 1,212 |
| E222K | 0.5 | 7.22 ± 0.75 | 108 ± 3 |  | 14,955 ± 1,618 |
| E222Q | 1.3 | 12.16 ± 0.65 | 668 ± 11 |  | 54,923 ± 3,084 |
| E222R | 0.15 | 2.48 ± 0.44 | 42 ± 2 |  | 17,098 ± 3,148 |
| E222Y | 0.7 | 18.43 ± 3.14 | 12 ± 1 |  | 636 ± 116 |
| R240A | 1.11 | 19.46 ± 1.17 | 11011 ± 258 |  | 565,763 ± 36,384 |
| R240D | 0.8 | 10.82 ± 0.47 | 282 ± 4 |  | 26,093 ± 1,196 |
| R240K | 1.4 | 17.67 ± 3.32 | 898 ± 59 |  | 50,829 ± 10,102 |
| I244E | 0.6 | 5.97 ± 1.04 | 497 ± 23 |  | 83,137 ± 14,963 |
| I244N | 0.21 | 2.15 ± 0.13 | 271 ± 4 |  | 126,176 ± 7,795 |
| M261E | 0.11 |  |  |  | 702 ± 73 |
| Q284R | 0.52 | 9.68 ± 1.35 | 370 ± 15 |  | 38,182 ± 5,550 |
| N293A | 0.68 | 9.67 ± 0.44 | 13 ± 0 |  | 1,313 ± 63 |
| Y294A | 0.59 | 4.98 ± 0.17 | 166 ± 2 |  | 33,260 ± 1,180 |
| Y294F | 0.73 | 5.99 ± 0.32 | 735 ± 11 |  | 122,751 ± 6,883 |
| Y295A | 0.69 |  |  |  | < 10 |
| Y295G | 0.77 |  |  |  | < 10 |
| T296A | 0.39 | 11.05 ± 0.77 | 109 ± 2 | 142.75 ± 28.67 | 9,904 ± 722 |
| S298E | 1.1 | 5.28 ± 0.05 | 809 ± 2 |  | 153,264 ± 1,391 |
| I300N | 1.51 | 4.48 ± 0.32 | 693 ± 13 |  | 154,732 ± 11,520 |
| Q313R | 1.07 | 3.58 ± 0.51 | 689 ± 24 |  | 192,373 ± 28,109 |
| H315N | ND |  |  |  |  |
| M323A | 0.35 | 9.34 ± 0.80 | 416 ± 11 | 126.29 ± 25.47 | 44,477 ± 3,991 |
| M323G | 0.88 | 19.21 ± 2.91 | 154 ± 9 |  | 7,998 ± 1,302 |
| M323K | ND |  |  |  |  |
| W325A | 0.26 | 1.61 ± 0.23 | 29 ± 1 | 171.02 ± 19.85 | 18,243 ± 2,607 |
| W325C | 0.22 | 4.18 ± 0.53 | 10 ± 0 | 159.19 ± 34.38 | 2,503 ± 327 |
| W325H | 1.12 | 3.08 ± 0.43 | 35 ± 1 | 143.45 ± 25.04 | 11,358 ± 1,645 |
| W325L | 1.08 | 5.74 ± 0.35 | 109 ± 2 |  | 18,909 ± 1,198 |
| P329W | ND |  |  |  |  |
| S331A | 0.89 | 4.34 ± 0.11 | 817 ± 5 |  | 188,306 ± 5,055 |
| K341A | 0.92 | 5.46 ± 0.33 | 1046 ± 17 |  | 191,689 ± 12,041 |
| T352A | 0.7 | 14.26 ± 1.76 | 60 ± 2 |  | 4,174 ± 541 |
| E353A | 0.56 |  |  |  | < 10 |
| N354A | 0.34 | 5.38 ± 0.67 | 3 ± 0 |  | 547 ± 70 |
| G355A | ND |  |  |  |  |
| M358T | 0.62 | 4.83 ± 0.48 | 436 ± 11 |  | 90,241 ± 9,225 |
| H373R | 1.19 | 6.31 ± 0.30 | 707 ± 9 |  | 112,169 ± 5,512 |
| H379R | 0.14 | 6.24 ± 0.84 | 2 ± 0 |  | 380 ± 53 |
| W399A | 0.96 | 16.65 ± 2.52 | 0 ± 0 |  | 14 ± 2 |
| W399C | 0.93 | 70.33 ± 5.89 | 3 ± 0 |  | 39 ± 4 |
| W399G | 1.27 |  |  |  | < 10 |
| W399S | 1.5 |  |  |  | < 10 |
| S400A | 0.41 | 3.22 ± 0.22 | 531 ± 9 |  | 164,795 ± 11,833 |
| D403A | ND |  |  |  |  |
| N404A | 1.41 | 9.42 ± 0.42 | 4 ± 0 |  | 393 ± 18 |
| F405A | ND |  |  |  |  |
| E406A | 0.57 |  |  |  | < 10 |
| E406D | 0.27 | 34.13 ± 2.57 | 39 ± 1 |  | 1,146 ± 94 |
| W407A | ND |  |  |  |  |
| W407G | ND |  |  |  |  |
| W407Q | ND |  |  |  |  |
| W407R | ND |  |  |  |  |
| W409A | ND |  |  |  |  |
| K413A | 1.11 | 2.92 ± 0.48 | 835 ± 33 |  | 285,858 ± 48,589 |
| F415A | 0.53 | 16.63 ± 4.00 | 1 ± 0 |  | 80 ± 20 |
| E423S | 1.08 | 6.60 ± 0.42 | 646 ± 12 | 317.35 ± 65.22 | 97,777 ± 6,431 |

**S1 Table: Kinetic constants for 103 computationally-designed BglB mutants.** Included are columns (1) the mutation (2) protein yield as assessed by absorbance at 280 nm, where ND indicates that the protein did not express above our limit of detection (3, 4, 5, 6) kinetic constants and nonlinear regression analysis for each of *k*cat, KM, KI, and *k*cat /KM.