|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| H-ras | Background corrected Kon (M-1 s-1)  H-ras/ Eu3+-GTP (10 nM)  (+GEF) | Background corrected Koff (s-1)  H-ras/ Eu3+-GTP (10 nM)  +100M GTP  (+GEF) | Kd (nM) derived from Koff/Kon  H-ras/ Eu3+-GTP  (+GEF) | Kd (nM)  mantGTPS (100 nM) / H-ras  (+EDTA, -Mg2+) | Kd (nM)  H-ras mantGTPS(100 nM) / RBD |
| wt | 3.76±0.11 \*105 | 0.00342±0.00009 | 9.1±0.4 | 207±19 | 417±52 |
| D47A/E49A | 4.29±0.17 \*105 | 0.00140±0.00011 | 3.3±0.3 | 854±110 | 636±98 |
| G48R | 3.76±0.11 \*105 | 0.00310±0.00005 | 8.2±0.3 | 251±19 | 534±74 |
| G48R/D92N | 3.36±0.08 \*105 | 0.00224±0.00004 | 6.7±0.2 | 439±46 | 410±80 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| H-ras | Kon  (normalized to wt)  H-ras/ Eu3+-GTP (10 nM)  (+GEF) | Koff  (normalized to wt)  H-ras/ Eu3+-GTP (10 nM)  (+GEF) | Kd derived from Koff/Kon  (normalized to wt)H-ras/ Eu3+-GTP  (+GEF) | Kd  (normalized to wt)  mantGTPS (100 nM) /H-ras  (+EDTA, -Mg2+) | Kd  (normalized to wt)  H-ras mantGTPS (100 nM)/ RBD |
| wt | 1.00±0.04 | 1.00±0.04 | 1.00±0.05 | 1.00±0.13 | 1.00±0.18 |
| D47A/E49A | 1.14±0.06 | 0.41±0.04 | 0.36±0.04 | 4.13±0.65 | 1.53±0.30 |
| G48R | 1.00±0.04 | 0.91±0.03 | 0.91±0.05 | 1.21±0.14 | 1.28±0.24 |
| G48R/D92N | 0.89±0.03 | 0.65±0.03 | 0.73±0.04 | 2.12±0.30 | 0.98±0.23 |

**Supplementary file 1. Thermodynamic and kinetic parameters of H-ras mutant *in vitro* experiments**