

Variant	T _{Growth} ^a	[E]	T _{Assay} (°C) ^b	<i>k</i> _{cat} (s ⁻¹) ^c	<i>K</i> _M (μM) ^c	<i>k</i> _{cat} / <i>K</i> _M (s ⁻¹ M ⁻¹) ^c
bbKSI	34°C	25-500nM	24.7 ± 0.5	1.7 ± 0.2	134 ± 54	(1.5 ± 0.9) × 10 ⁴
tKSI	29°C	0.5-5nM	26.0 ± 1.6	24.9 ± 12.2	40 ± 16	(6.1 ± 1.1) × 10 ⁵
mbKSI	46°C	5-50nM	25.1 ± 0.1	6.9 ± 0.6	40.5 ± 12	(1.8 ± 0.4) × 10 ⁵
mhKSI	37°C	10-500nM	25.2 ± 0.2	1.3 ± 0.1	92 ± 20	(1.5 ± 0.2) × 10 ⁴
miKSI	37°C	5-100nM	24.7 ± 0.8	1.6 ± 0.5	65 ± 37	(2.7 ± 0.9) × 10 ⁴
mmKSI	31°C	0.25-5nM	25.6 ± 1.3	106.3 ± 21.0	45 ± 21	(3.3 ± 2.7) × 10 ⁶
mpKSI	37°C	10-50nM	25.9 ± 1.4	3.4 ± 0.5	76 ± 28	(4.7 ± 1.0) × 10 ⁴
msKSI	37°C	5-50nM	25.0 ± 0.1	29.2 ± 7.7	80 ± 12	(3.7 ± 1.1) × 10 ⁵
mtKSI	39°C	0.5-25nM	26.0 ± 1.3	44.3 ± 7.7	47 ± 4	(9.6 ± 2.1) × 10 ⁵
naKSI	29°C	10-50nM	27.1 ± 0.6	15.9 ± 3.1	144 ± 52	(1.2 ± 0.2) × 10 ⁵
ntKSI	29°C	10-100nM	26.5 ± 1.2	4.8 ± 1.1	104 ± 49	(5.0 ± 1.0) × 10 ⁴
npKSI	20°C	25-250nM	24.8 ± 0.4	6.8 ± 2.0	368 ± 31	(1.8 ± 0.4) × 10 ⁴
oiKSI	29°C	1-40nM	25.9 ± 0.9	19.2 ± 7.6	107 ± 41	(2.6 ± 1.4) × 10 ⁵
psKSI	15°C	50-500nM	25.0 ± 0.0	1.7 ± 0.0	411 ± 30	(4.1 ± 0.3) × 10 ³
pgKSI	20°C	0.5-25nM	26.3 ± 0.9	18.1 ± 8.2	109 ± 86	(2.6 ± 2.2) × 10 ⁵
paKSI	34°C	0.5-15nM	26.8 ± 0.5	9.4 ± 2.3	21 ± 18	(7.7 ± 5.9) × 10 ⁵
pKSI	28°C	1.5-15nM	25.7 ± 1.1	11.2 ± 3.1	25 ± 13	(5.5 ± 2.7) × 10 ⁵
rmKSI	18°C	5-50nM	25.0 ± 0.0	10.3 ± 2.7	125 ± 13	(8.2 ± 1.2) × 10 ⁴
ssKSI	16°C	5-50nM	24.6 ± 0.6	8.7 ± 2.5	19 ± 3	(4.4 ± 0.7) × 10 ⁵
spKSI	20°C	0.5-10nM	26.4 ± 0.4	70.1 ± 21.6	77 ± 27	(9.8 ± 3.4) × 10 ⁵

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51 **Figure 3—source data 2: Kinetic measurement of KSIs at 25°C with substrate 5(10)-estrene-3,17-dione.**

52 ^a (Engqvist, 2018)

53 ^b Reported assay temperatures are the average of at least three measurements per experiment.

54 ^c average ± standard deviation from 2–9 independent experiments with enzyme concentration varied by at least 5-fold.

55 Values measured with substrate concentrations from 9-600 μM. Value of *k*_{cat}/*K*_M are less than 10⁷ M⁻¹ s⁻¹ and thus

56 unlikely to be limited by substrate binding. Reported assay temperatures are the average of at least 3 measurements

57 per experiment.