

Table S1. Steady-state parameters for activation of various isoleucine samples by WT EcLeuRS^a^a Measured by ATP-PP_i exchange assay.

AA	% Leu ^b	<i>K_m</i> (AA)	<i>k_{cat}</i>	<i>k_{cat}/K_m</i>	Discrimination factor ^c
		mM	s ⁻¹	mM ⁻¹ s ⁻¹	
commercial Ile (Sigma BioUltra lot 1)	0.38 ± 0.03	5.0 ± 0.4	65 ± 2	13	102
commercial Ile (Sigma BioUltra lot 2)	(19 ± 2) × 10 ⁻⁴	23	3.8	0.17	7765
purified Ile (Sigma BioUltra lot 2)	(3.5 ± 0.7) × 10 ⁻⁴	26 ± 7	1.1 ± 0.1	0.042	31429

^b Determined kinetically- see Supplementary Materials and Methods.^c Discrimination factor is defined as (*k_{cat}/K_m*)_{cognate}/(*k_{cat}/K_m*)_{non-cognate}.

The values represent the best fit value ± S.E. of at least two independent experiments.