

**Table 3. Kinetic and thermodynamic parameters for non-interacting mutants of IFN $\alpha$ 2 – ifnar2**

ifnar2	IFN $\alpha$ 2	$k_d^a$ (s <sup>-1</sup> )	$\Delta\Delta G^b$ (kJ/mol)	$K_D^c$ ( $\mu$ M)	$\Delta\Delta G^d$ (kJ/mol)
T46A	L15A			1.05	-14.5
T46A	L26A			0.77	-13.8
T46A	F27A			0.41	-12.3
T46A	L30A			58.6	-24.2
T46A	R144A			2.32	-16.5
T46A	A145G			3.87	-17.7
T46A	M148A			4.68	-18.1
T46A	R149A			18.2	-21.4
T46A	S152A			0.44	-12.4
T46A	L153A			0.67	-13.5
I47A	L15A			-15.3	
I47A	L26A			-14.4	
I47A	F27A			-13.2	
I47A	L30A			-23.6	
I47A	R144A			3.38	-17.4
I47A	A145G			4.76	-18.2
I47A	M148A			3.18	-17.2
I47A	R149A			11.6	-20.3
I47A	S152A			0.40	-12.2
I47A	L153A			1.02	-14.5
K55A	R12A	0.059	-4.1		
K55A	L15A	0.147	-6.4		
K55A	L26A	0.128	-6.1		
K55A	F27A	0.052	-3.8		
K55A	D35A	0.032	-2.6		
K55A	K133A	0.045	-3.5		
K55A	A145G	0.401	-8.9		
K55A	S152A	0.118	-5.9		
K55A	L153A	0.298	-8.1		
W102A	R12A			0.13	-9.4
W102A	L15A			0.45	-12.5
W102A	M148A			1.65	-15.6
W102A	S152A			0.26	-11.2
W102A	L153A			0.36	-12.0
I105A	WT			0.04	-7.5
I105A	L15A			1.38	-15.8
I105A	L26A			0.42	-13.0
I105A	F27A			0.29	-12.1
I105A	L30A			24.7	-22.8
I105A	R144A			0.96	-14.9
I105A	M148A			2.27	-17.0
I105A	R149A			9.91	-20.6
I105A	S152A			0.61	-13.9
I105A	L153A			0.71	-14.2
D106A	R12A	0.112	-5.7		
D106A	L26A	0.246	-7.7	0.36	-12.0
D106A	F27A	0.053	-3.9	0.14	-9.8
D106A	R144A	0.160	-6.6	1.23	-14.9
D106A	S152A	0.100	-5.4	0.23	-10.9
D106A	L153A	0.314	-8.3	0.41	-12.3

All measurements were done using RifS.

- <sup>a</sup> Dissociation rate constant.
- <sup>b</sup>  $\Delta\Delta G$  was calculated from  $k_d$  according to eq. 2.
- <sup>c</sup> Values of  $K_D$  were determined from the equilibrium response guided by the law of mass-action.
- <sup>d</sup>  $\Delta\Delta G$  was calculated from  $K_D$ .