

Table 1: Reductase and esterase activities of wildtype and mutant *E. coli* GluTRs with different substrates *in vitro*. The activity of GluTR variants are provided relative to that of wildtype GluTR (100%). The specific activity for wildtype GluTR was 0.1  $\mu\text{mol min}^{-1} \text{mg}^{-1}$  with Glu-tRNA<sup>Glu</sup> and 1.8  $\text{nmol min}^{-1} \text{mg}^{-1}$  with 4-nitrophenyl acetate as substrate. n.d., not detectable, n.t., not tested, \* data from [14]. A standard deviation of less than 10% was observed, except where stated otherwise.

Enzyme	Activity with Glu-tRNA <sup>Glu</sup> [%]		Activity with Gln-tRNA <sup>Glu</sup> [%]	Esterase activity with 4-nitrophenyl acetate [%]
	Reductase	Esterase		
wt	100	100	15	100 ± 9
S109A	28	25	n.d.	102 ± 11
T49V	10	5	n.d.	94 ± 16
H99N	5	4	n.t.	68 ± 14
E54K	6	2	n.t.	105 ± 8
R52K	5	4	n.d.	62 ± 16
R52Q	n.d.	n.d.	n.d.	68 ± 9
E114K*	n.d.*	n.d.*	n.t.*	n.t.*
C50S	n.d.	n.d.	n.t.	n.d.
Q116L	n.d.	30	n.t.	93 ± 2