



Figure S2. A kinetic assay was used to determine the percentage of leucine in isoleucine samples. A, Representative plateau formation of Leu-tRNA^{Leu} at various concentrations of isoleucine – 20 mM (●), 40 mM (▲), 60 mM (◆). WT LeuRS (additionally purified to remove Leu-AMP bound in the enzyme's active site) was present at 4 μM concentration and tRNA^{Leu} was present at 20 μM concentration. B, Replot of Leu-tRNA^{Leu} plateaus vs isoleucine concentration. The slope of the line represents the fraction of leucine present in L-Ile Sigma BioUltra lot 1 (◆), L-Ile Roth Cellpure (◇), L-Ile Serva Analytical grade (▼), and L-Ile Sigma BioUltra lot 2 unpurified (○) or purified (●). The *inset* shows Leu-[³²P]-tRNA^{Leu} formation in dependence of L-Ile BioUltra lot 1 concentration on a plot with a narrower x-axis scale.