

Primers / Probe titration and ratio

The commonly used concentrations for primers and for probes are 300 nM and 100 nM respectively. Optimal results may require titration of primers and probes or adjustment of the primer / probe ratio. The purpose of such a process is to determine the minimum amount of primers and probe required to obtain the most sensitive results with your assay.

Primer titration matrix

Titrate according to the Table 1, perform qPCR and select the concentration which gives the lowest C_q value. By doing this type of titration it is also possible to compensate for differences up to 2 °C in melt temperature of the primers.

Reverse	Forward		
	50 nM	300 nM	900 nM
50 nM	50 / 50	300 / 50	900 / 50
300 nM	50 / 300	300 / 300	900 / 300
900 nM	50 / 900	300 / 900	900 / 900

Table 1: Primer titration matrix

Primer-probe ratio matrix

Select optimal primer concentration as described in Table 1 and test with all probe concentrations described in Table 2. Select the concentration which gives the lowest C_q value.

Primers	Probe		
Opt. primers concentration	50 nM	100 nM	250 nM

Table 2: Primer-probe ratio matrix