Absolute Standard Curve Method versus Relative Standard Curve Method

The standard curve method in real-time quantitative PCR can be used to determine either the absolute quantity (AQ) or the relative quantity (RQ) of target sequence. This quick reference highlights the differences between the methods. A schematic diagram is provided to illustrate how relative target quantity is determined by the software in an easy-to-understand manner.

	Absolute Standard Curve Method[1]	**Relative Standard Curve Method[1]**
Application	Determine absolute target quantity in samples (such as mass, molarity, or viral load)	Determine relative target quantity in samples. The method is commonly used to: a. Compare expression levels of a gene in different tissues b. Compare expression levels of a gene in a treated sample and an untreated sample c. Compare expression levels of wild-type alleles and mutated alleles d. Analyze the gene expression changes over time under specific treatment conditions
Experiment type selection in instrument software*	Standard Curve, Quantitation-Standard Curve, Standard Curve (AQ)	Relative Standard Curve, Quantitation-Relative Standard Curve
Sample type for creating standard curve	A dilution series of target RNA or DNA with known quantities	Any type of sample (plasmid, RNA, or DNA) that contains the target sequence for gene of interest (GOI) and for endogenous control (EC)
Requirement of endogenous control	No	Yes
Requirement of reference sample	No	Yes
Data analysis	The software measures amplification of the target in samples and in a standard dilution series of a control sample. Data from the standard dilution series are used to generate the standard curve. Using the standard curve, the software interpolates the absolute quantity of target in the samples.	Software measures amplification of the target and of the endogenous control (EC) in samples, in a reference sample, and in a standard dilution series. Measurements are normalized using the EC. Data from the standard dilution series are used to generate the standard curve. Using the standard curve, the software interpolates target quantity and EC quantity in the samples and in the reference sample. For each sample and reference sample, the target quantity is normalized by EC quantity (quantity of target/quantity of EC). The normalized quotient from samples is divided by the quotient from the reference sample to get relative quantification (fold change). The software determines the relative quantity of target in each sample by comparing target quantity in each sample to target quantity in the reference sample.

* Applied Biosystems

ThermoFisher SCIENTIFIC

An Example of Relative Standard Curve Experiment:

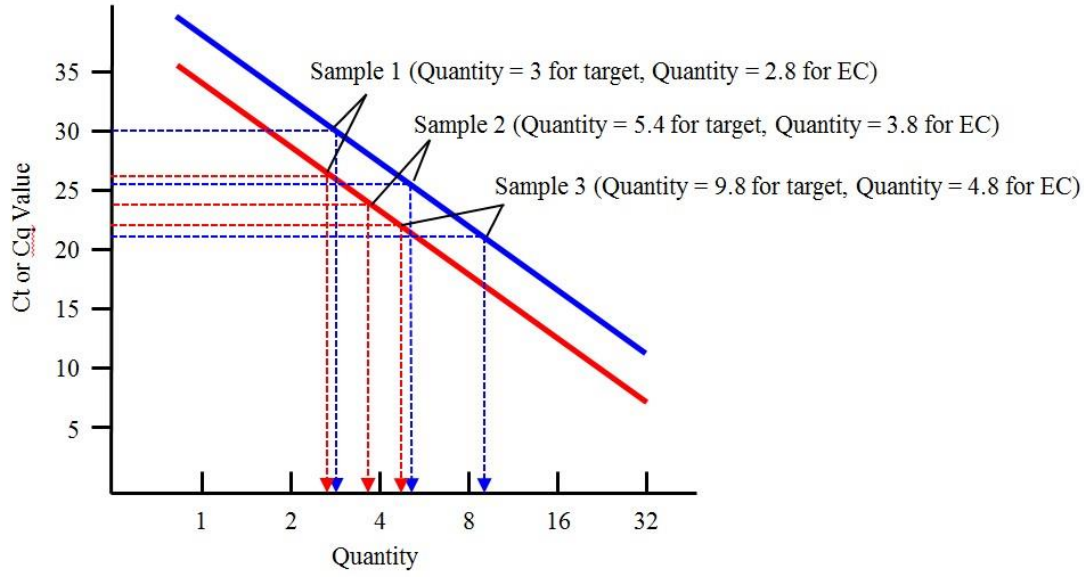

Figure: Relative Standard Curve Plot. Solid line in blue represents standard curve plot for target. Solid line in red represents standard curve plot for endogenous control (EC). Software uses the standard curve plot to interpolate the target quantity and the quantity of EC in three samples.

	Sample 1 (reference)	Sample 2	Sample 3
Quantity for Target	3.0	5.4	9.8
Quantity for EC	2.8	3.8	4.8
Normalized Quantity for Target[a]	1.1	1.4	2.0
Relative Quantity for Target[b]	1	1.3	1.8

Table: Calculation of Relative Quantity (RQ) of Target Sequence. a. Normalized quantity of target is calculated by dividing the quantity of target with the quantity of EC; b. Sample 1 is selected as the reference sample. The relative target quantity in sample 2 and sample 3 is determined by dividing normalized target quantity in sample 2 and sample 3 with normalized target quantity in the reference sample, respectively.

Reference:

1. QuantStudio 6 and 7 Flex Real-Time PCR System Software Getting Started Guide. Applied Biosystems (2013)