Quantification of Nucleic Acid Yield

The UV-absorbance quantification method uses a spectrophotometer to measure the natural absorbance of light at 260 nm (for DNA and RNA) or 280 nm (for proteins). Because the Arcis kit extracts total nucleic acids and proteins that absorb light at 260 nm, this measurement cannot be used to quantify DNA or RNA.

1. Qubit™ fluorometric quantification

Qubit™ fluorometric quantification of DNA, RNA, protein and microRNA is based on the detection of target-specific fluorescence, this integrated system is more sensitive than UV absorbance–based quantification. Each dye is specific for one type of molecule: DNA, RNA or protein. These dyes have extremely low fluorescence until they bind to their targets (DNA, RNA or protein). Upon binding, they become intensely fluorescent.

- Rapid detection of target in 1-20μl sample.
- Use of dyes selective for dsDNA, RNA, or protein minimizes the effects of contamination in the sample.
- After calibration of the instrument, and reading standards a fluorescence v concentration graph will appear on the screen.
- Samples are then run after selection volume sample(μl) and output units (eg. ng/μl).
- Samples are then read.

2. Quantification of template using CT.

qPCR enables you to determine the starting template copy number.

The sample amplification plot of amount of product (fluorescence) against number of cycles has an exponential phase and a non-exponential phase. During the exponential phase the amount of product doubles with each cycle.

Initially fluorescence remains at a background level, but eventually the amount of amplified product accumulates enough to yield a signal. The cycle number at which this occurs is call the CT value (threshold cycle). If a large amount of template is present at the start of a reaction the CT will be low or early.

CT can be used to quantify the template by comparing the PCR CT with the CT obtained for a genomic DNA reference gene (such as hRNaseP) and directly comparing the CT using the equation:
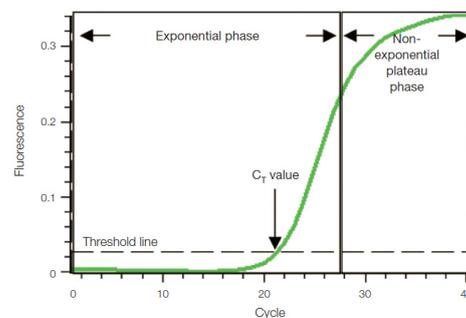
Ratio (reference/target)= 2 CT(reference)- CT (target).

Fig. 1.1. Amplification plot. Baseline-subtracted fluorescence is shown.

For further information please visit **arcisbio.com**

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