Lab Manager

DIGITAL PCR

ABSOLUTE QUANTIFICATION OF TARGET NUCLEIC ACIDS USING PARTITIONING

Digital polymerase chain reaction (dPCR) enables absolute quantification of target nucleic acids in a sample. The sample is partitioned into many individual reactions, each containing only a few target sequences. Following PCR amplification, amplified target sequences are detected by fluorescence and the ratio of positive partitions to total partitions is used to determine the concentration of the target sequence in the sample. Quantification is based on the random distribution of molecules in multiple partitions that follows a Poisson distribution.

d-PCR ADVANTAGES

- ☑ sample partitioning concentrates target sequences and reduces template competition
- ☑ dPCR does not rely on calibration curves for sample quantification
- ☑ offers a higher tolerance to inhibitors present in samples
- ☑ improved detection of low-copy number variants

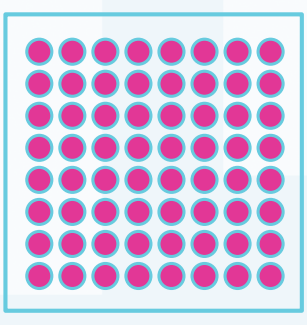
HOW IT WORKS

SAMPLE PREPARATION

PCR solution containing template, fluorescence-quencher probes, primers, PCR master mix (DNA polymerase, dNTPs, $MgCl_2$, and reaction buffers).

PARTITIONING

The sample is partitioned into individual sub-reactions, using microfluidic chamber-based, micro-well chip-based, or droplet-based methods.

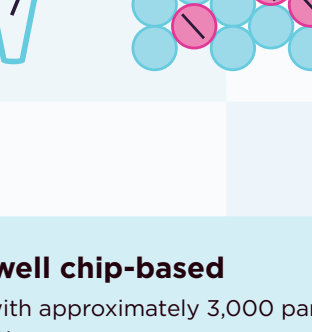


AMPLIFICATION

Target sequences are amplified within each individual partition.

DETECTION

Target sequences are detected by fluorescence.



PARTITIONING TECHNOLOGIES

Microfluidic chamber-based

Up to several hundred partitions per panel

Droplet-based

Approximately 20,000 and up to 10,000,000 partitioned droplets per reaction

Microwell chip-based

Plates with approximately 3,000 partitions per array