

PROBES AND ASSAYS FOR DIGITAL PCR (dPCR)

Cost-effective and conveniently packaged reagents for genotyping and gene expression applications using dPCR



Convenient packaging—probes provided in 0.5–2.5 nmol amounts



Fast turnaround time—start your experiments sooner



More economical pricing—stretch your budget

Digital PCR (dPCR) is a quantitative PCR method that provides a sensitive, reproducible method for quantifying the amount of DNA or RNA present in a sample. It utilizes similar assay reagents as in standard qPCR measurements, but instead of analyzing the whole reaction, dPCR separates the mixture into individual nanoliter reactions (Figure 1). The final analysis counts the total number of individual partitions containing target molecules, providing an absolute measurement of the expression for the gene of interest. Digital PCR enables many applications that require high sensitivity and have limited sample availability. dPCR is amenable to many applications, including rare allele detection, liquid biopsy analysis, viral load detection, single-cell analyses, and DNA quality control for sequencing.

IDT offers a variety of probes and assays for genotyping and gene expression applications using microwell- or droplet-based dPCR platforms.

Sample preparation

gDNA or cDNA
Primers and 5' nuclease probe
Master mix

Dilution & partition

PCR amplification & end point analysis

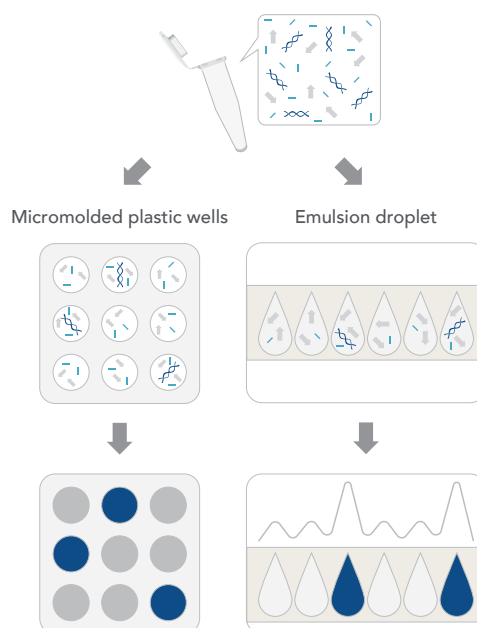


Figure 1. Digital PCR (dPCR) workflow.

After isolation of genomic DNA (gDNA) or conversion of an mRNA sample to cDNA, master mix, target specific primers, and 5' nuclease probe(s) are added. The mix is diluted and partitioned so there is either 1 or 0 targets in each partition. Some dPCR platforms partition into micromolded plastic wells; whereas, other dPCR platforms create emulsions of oil and water droplets. After PCR amplification, the fluorescent signal for each well or droplet is recorded. The number of positive wells or droplets provides an accurate count of the number of starting target gDNA or cDNA in the sample.

SNP GENOTYPING APPLICATIONS

Mini Affinity Plus™ qPCR Probes containing locked nucleic acids are ideal for SNP genotyping, transcript variant identification, and more sensitive target detection in challenging samples (FFPE tissue, biofluids). When incorporated into a probe, locked nucleic acids impart heightened structural stability, leading to increased hybridization melt temperature (T_m). The Affinity Plus qPCR probes include up to 6 Affinity Plus monomers allowing flexible T_m adjustments (Table 1). Mini Affinity Plus qPCR Probes are ideal for dPCR applications, screening small sample sets, or performing just a few reactions when optimizing probe designs. Whether used in simplex or 4-plex assays, Affinity Plus qPCR probes demonstrate equal sensitivity for detection of *CYPC219* quad-allele single nucleotide variations (Figure 2, data courtesy of Combinati). (Shipped in 4–7 business days.)

Table 1. Mini Affinity Plus qPCR Probes

| 5' reporter dye(s) | Emission (nm) | Quencher(s) | Delivery amount |
|--------------------|---------------|----------------|-----------------|
| FAM | 520 | | |
| SUN™ | 554 | lowa Black™ FQ | 0.5 nmol |
| HEX | 555 | | |
| Cy®5 | 668 | lowa Black RQ | |

For more information, to view larger delivery amounts (8 and 20 nmol minimum guarantee), or to place an order, go to www.idtdna.com/AffinityProbes. For information on designing or ordering probes labeled with ATTO or other fluorophores not listed, contact us at www.idtdna.com/affinity-support.

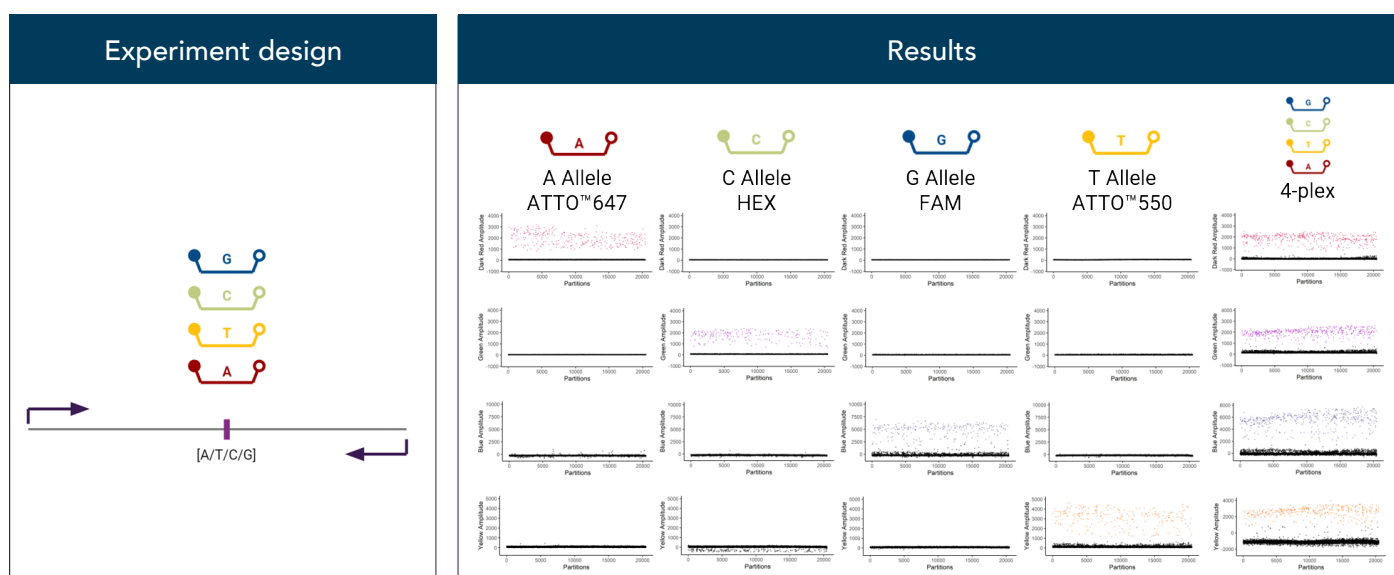


Figure 2. 4-color Affinity Plus qPCR Probe multiplex assay for the discrimination of quad-allele single nucleotide variation (SNV) target on the Absolute Q™ (Combinati). This multiplex probe assay targets a single SNV location and identifies each of the 4 possible alleles. For this study, gBlocks™ Gene Fragments containing each of the 4 target sequences were combined to create a pooled sample. Each assay was tested in simplex using the pooled control gBlocks fragments to demonstrate specificity (columns 1–4). The assays were then combined as a single-tube 4-plex assay (column 5). The data for this assay clearly demonstrate that there is no loss of sensitivity for 4-plex reaction in comparison to simplex assays. Data courtesy of Combinati.

GENE EXPRESSION APPLICATIONS

PrimeTime™ qPCR Probes provide reliable, high-quality gene expression data. These 5' nuclease probes are available with an assortment of reporter-dye combinations compatible with common qPCR instruments. ZEN™ and TAO™ Double-Quenched Probes from IDT reduce background fluorescence for longer probe designs. Reduced background improves accuracy of sensitive dPCR applications. Choose from a variety of synthesis scales, dyes, and shipping options to meet your needs. Estimated turnaround times depend on the degree of probe complexity (Table 2). The Mini and Eco sizes are ideal for screening small sample sets, or performing just a few reactions when optimizing probe designs. Larger scales are also available with customizable ratios of probes and primers. (Mini/Eco delivery amounts ship within 3–5 days, and other scales ship within 5–7 business days.)

Table 2. Primetime qPCR Probes—small scale synthesis

| 5' reporter dye | Emission (nm) | Quencher(s) | Delivery amount | | Minimum guarantee |
|-----------------|---------------|-------------------|-----------------|----------------|---------------------|
| | | | Mini (0.5 nmol) | Eco (2.5 nmol) | Other scales (nmol) |
| FAM | 520 | ZEN/Iowa Black FQ | √ | √ | 15, 25, 50 |
| SUN | 554 | ZEN/Iowa Black FQ | √ | √ | 15, 25, 50 |
| HEX | 555 | ZEN/Iowa Black FQ | √ | √ | 10, 25, 50 |
| Cy 5 | 668 | TAO/Iowa Black RQ | √ | √ | 2, 8, 20 |

For more information, to view additional yield guarantee sizes, or to place an order, go to www.idtdna.com/qPCRprobes.

PrimeTime qPCR Probe Assays (Mini). This unit size is used for dPCR, screening small sample sets, or performing just a few reactions when optimizing probe designs. These assays consist of a primer pair and 5' nuclease probe, and are available in various sizes, premixed, and shipped dried down in either tubes or plates (Tables 3 and 4). The probes/primers are supplied in the following ratio: 0.5/1.0 nmol. Larger unit sizes are available. Assays in tubes ship within 2–4 business days. Assays in plates ship within 5–9 business days. Minimum order of 24 assays is required per plate.

Table 3. PrimeTime Probe Assays in tubes

| 5' reporter dye | Emission (nm) | Quencher(s) | Unit size | Reactions | Other reaction sizes |
|-----------------|---------------|-------------------|-----------|-----------|----------------------|
| FAM | 520 | ZEN/Iowa Black FQ | Mini | 100 | 500, 2500 |
| SUN | 554 | ZEN/Iowa Black FQ | | | |
| HEX | 555 | ZEN/Iowa Black FQ | | | |
| Cy5 | 668 | TAO/Iowa Black RQ | | | |

Table 4. PrimeTime Probe Assays in 96-well plates

| 5' reporter dye | Emission (nm) | Quencher(s) | Unit size | Reactions | Other reaction sizes |
|-----------------|---------------|-------------------|-----------|-----------|----------------------|
| FAM | 520 | ZEN/Iowa Black FQ | Mini | 100 | 500, 2500 |

For more information, to view larger unit sizes, or to place an order, go to www.idtdna.com/primetime-probe-assays.

Table 5. Digital PCR instrument compatibility and reporter dyes

| | Max emission wavelength (nm) and dye | | | | | | | | | | | |
|---------------------------|--------------------------------------|------|------|-----|------|-------|------------|-----|--------------|------------|------|--------|
| | 520 | 554 | 555 | 555 | 557 | 564 | 575 | 608 | 617 | 662 | 668 | 706 |
| Instrument | 6-FAM | SUN™ | JOE™ | HEX | MAX™ | Cy® 3 | ATTO™ 550# | ROX | Texas Red®-X | ATTO 647N§ | Cy 5 | Cy 5.5 |
| Applied Biosystems | | | | | | | | | | | | |
| QuantStudio® 3D | • | • | • | • | x | x | x | •* | x | x | x | x |
| Bio-Rad | | | | | | | | | | | | |
| QX100™ | • | • | • | •1 | x | x | x | x | x | x | x | x |
| QX200™ | • | • | • | •1 | x | x | x | x | x | x | x | x |
| QX One™ | • | • | • | •1 | x | x | x | x | x | • | • | • |
| Stilla | | | | | | | | | | | | |
| Naica™ | • | • | • | • | • | • | • | • | x | • | • | • |
| Combinati | | | | | | | | | | | | |
| Absolute Q™ | • | • | • | • | • | • | • | # | # | • | • | x |
| Fluidigm | | | | | | | | | | | | |
| Biomark | • | • | • | • | • | • | x | • | • | x | x | x |
| Dropworks | | | | | | | | | | | | |
| Continuum™ | • | • | • | • | x | x | x | • | x | • | • | x |

- Supplier provided or recommended reporter dyes
- Instrument capable dyes, but may require calibration
- x Instrument incapable of supporting
- # Instrument uses channel for reference dye
- * Cannot be used if ROX is used as a passive reference dye
- # Preferred dye equivalent for TAMRA
- § Preferred dye equivalent for Cy 5

> FOR MORE INFORMATION, VISIT WWW.IDTDNA.COM.

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