Dark Probe Mutation Detection Assays

More Targets. Less Sample. Higher Sensitivity.

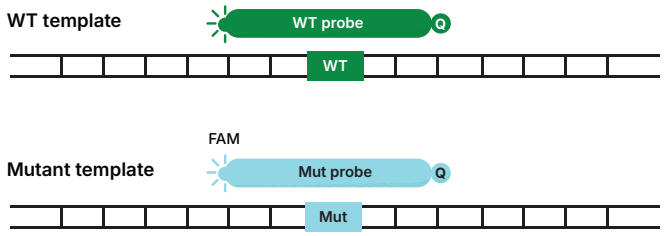
Dark Probe assays decouple wild-type suppression from fluorescence, freeing detection channels on Bio-Rad Droplet Digital™ PCR (ddPCR™) Systems.* Detect more mutations per reaction, and include reference genes for accurate VAF calculation, without increasing sample input or sacrificing sensitivity.

How It Works

Replace the wild-type fluorescent probe with a quencher-only dark probe to maintain specificity while freeing each detection channel for an additional mutation target or reference gene. The freed channels can be used to increase assay multiplexing, incorporate reference genes for VAF normalization, or both, all from the same sample input.

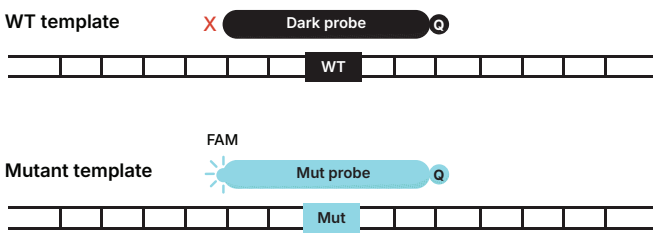
Standard vs. Dark Probe Approach

Standard mutation assay



- 2 fluorescent probes (wild-type and mutant)
- 2 channels per mutation
- Maximum 3 mutations per well

Dark probe approach



- Non-fluorescent WT probes
- 1 channel per mutation target
- Up to 4 mutations and 2–3 reference genes per well (system-dependent)
- Include VAF reference genes (e.g., *RPP30*, *AGO1*) without a separate reaction

Get More Targets From Every Well

Instrument	Standard Mutation Detection Assays	Dark Probe Mutation Detection Assays
QX200™ ddPCR System†	1 mutation and 1 WT per well	2 mutations or 1 mutation and 1 reference gene per well
QX600 ddPCR System	3 mutations per well	4 mutations and 2 reference genes per well
QX700™ ddPCR System*	3 mutations per well	4 mutations and 3 reference genes per well

Reference genes (e.g., *RPP30*, *AGO1*) can be included in freed channels for VAF/FA% normalization. No additional reaction required.

Key Benefits

Higher multiplex density: detect up to four mutations plus up to three reference genes in a single well (system-dependent), without increasing sample input.

Lower cost per target: consolidate panels into one well to reduce wells, consumables, reagent volume, and preserve precious sample.

Flexible panel design: no required fluorophore pairings (FAM/HEX, Cy5/Cy5.5, ROX/ATTO). Freely assign channels to mutations or reference genes based on your panel design. Order through the Bio-Rad Mutation Detection Design Engine.

Available Formats

Channels: FAM, HEX, Cy5, Cy5.5 Reaction sizes: 200 x 20 µL, 1,000 x 20 µL, 2,500 x 20 µL

* For Research Use Only. Not for use in diagnostic procedures.
† For Research Use Only. In vitro diagnostic (IVD) option is available.

Common Applications

Oncology research: mutation profiling panels for *EGFR*, *KRAS*, *BRAF*, and other variants across tissue, FFPE, and ctDNA samples.

MRD and liquid biopsy: track rare variants and monitor therapy responses at low VAF in ctDNA.

Translational research: confirm NGS calls and quantify SNVs in a single reaction.

Performance At a Glance

Sensitivity: 0.05–0.1%† VAF at 100 ng input per well; lower VAF achievable with increased input or multiple wells.

Multiplexing: six targets: four mutations and two reference genes can be resolved in a single well (Figure 1).

† Achievable sensitivity is driven by sample input, target abundance, and assay design.

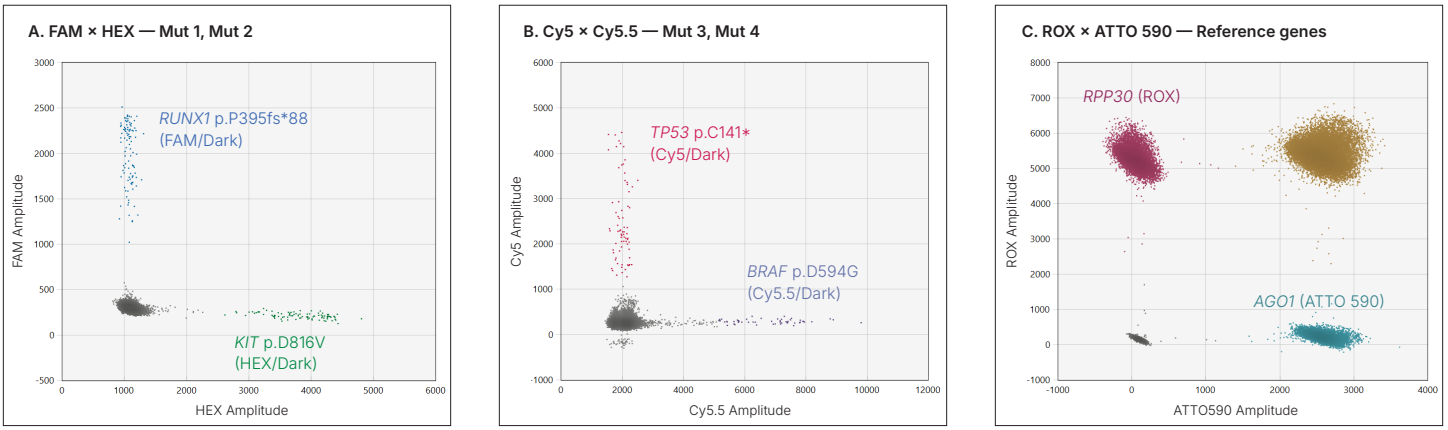


Fig. 1. Multiplex detection of four mutations and two reference genes in a single QX600 ddPCR System well. Six targets resolved across three channel pairs in a single QX600 ddPCR System well: four mutations in panels **A** and **B** (with dark probes suppressing wild-type signal), and two reference genes co-amplifying in panel **C** for normalization.

Ordering Information

Channel	Description	200 x 20 µL Reactions	1,000 x 20 µL Reactions	2,500 x 20 µL Reactions
FAM	ddPCR MUT FAM+Dark Assay	12016634	12016618	12016619
HEX	ddPCR MUT HEX+Dark Assay	12016607	12016586	12016617
Cy5	ddPCR MUT Cy5+Dark Assay	12016608	12016638	12016624
Cy5.5	ddPCR MUT Cy5.5+Dark Assay	12016635	12016636	12016637

ROX and ATTO 590 assays: coming soon. All Dark Probe assay sequences are designable via the Bio-Rad Mutation Detection Design Engine.

Visit **bio-rad.com/MutationDetection** for more information.

Bio-Rad Laboratories, Inc.

Life Science Group

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