

A Practical Guide to Running Your First Dark Probe Assays Experiment

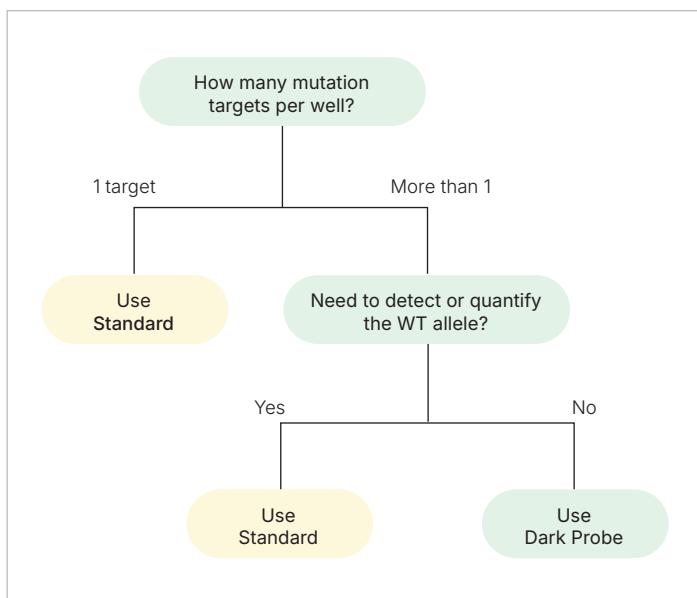
Getting Started with Dark Probe Mutation Detection Assays

Dark probe assays replace the wild-type fluorescent probe with a quencher-only dark probe, suppressing wild-type background while freeing a detection channel. This enables higher-order multiplexing by allowing more mutation targets to be analyzed in a single well. This guide walks you through selecting the right assay configuration, designing a multiplex panel, and planning a successful experiment.

1 Choose Your Configuration

It comes down to how many targets you're detecting and whether you need the wildtype allele itself.

The **dark probe** configuration frees the wild-type detection channel, so more mutation targets fit in a single well.



2 Build Your Panel in 3 Easy Steps

1. Visit the Assay Design Engine
Go to bio-rad.com/MutationDesign
2. Enter your targets
Input mutations, select fluorophores (FAM/Dark, HEX/Dark, Cy5/Dark, Cy5.5/Dark)
3. Place your order
Review, select reaction size (200, 1,000, 2,500), and submit

Enter Target by: Identifier Sequence	Fluorophore: Mutant/Wildtype	Tube Format
Identifier: ESRR p.ETNA_A750G Sequence: TAACTGG (single tube only)	TAACTGG (single tube only)	Single Tube
Identifier: ESRR p.L588R c.2372_2374TG>GT Sequence: TAACTGG (single tube only)	HEX/Dark (single tube only)	Single Tube
Identifier: COSMID8748 Sequence: TAACTGG (single tube only)	Cy5/Dark (single tube only)	Single Tube
Identifier: KRAS p.G12C c.343G>T Sequence: TAACTGG (single tube only)	Cy5.5/Dark (single tube only)	Single Tube

Key Features

Three things that let dark probe assays drop straight into your existing Droplet Digital™ PCR (ddPCR™) workflow.



Instrument Compatibility Across the QX Series

Fully compatible across QX series ddPCR systems—no hardware changes.



Multiplexing Capability: Up to 4 Mutant Targets per Well

Single-well multiplexing on the QX600* and QX700* Systems—so you analyze more mutations per microliter of precious sample.



Wild-Type Suppression Quencher-Only Dark Probe

Enhanced suppression of the wild-type allele without spending a detection channel.

* For Research Use Only. Not for use in diagnostic procedures.



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

Frequently Asked Questions

Common Questions About Dark Probe Assays

Everything you need to know about compatibility, thresholds, signal balance, and troubleshooting.

Q: Can I run Dark Probe assays on my instrument?

Yes. Dark probe assays are compatible across the Bio-Rad QX Series—QX200™, QX ONE™, QX600, and QX700 ddPCR Systems—using standard run conditions. Up to four mutant targets per well are supported on the QX ONE, QX600, and QX700 ddPCR Systems; the two-channel QX200 System supports a two-mutation duplex.

Q: How much DNA can I load per reaction?

Dark probe assays are tested for inputs up to 100 ng per reaction, which supports high-input applications such as FFPE, high-cellularity tissue, and enriched cfDNA. Higher input increases sensitivity (more mutant copies sampled) but also raises wild-type signal—if background creeps up, reduce input or confirm your threshold against a wild-type-only well.

Q: Signal in my reference channels is too bright.

What should I do?

ROX and ATTO 590 dyes are intrinsically brighter than FAM, HEX, Cy5, and Cy5.5. If the reference clusters are oversaturated, reduce the reference assay to 0.3–0.5x the standard concentration per reaction. This pulls the reference amplitude back into range without affecting the mutation channels.

Q: Are my assays compatible for multiplexing?

Ensure your assays do not target overlapping sequences by checking the MIQE coordinates. If they do, do not combine them in the same well—instead, split them across separate wells or reach out to your Bio-Rad Field Application Specialist for custom assay design assistance. This prevents probe competition and ensures accurate cluster separation. Note that some assays may show suboptimal cluster separation in the Cy5 and Cy5.5 channels due to dye-specific performance variability. If you observe this, consider reassigning those challenging targets to the FAM or HEX channel. Redesign the affected assay in the FAM/Dark or HEX/Dark format via the Mutation Detection Design Engine and adjust your multiplex layout accordingly.

* For Research Use Only. In vitro diagnostic (IVD) option is available.

† For Research Use Only. Not for use in diagnostic procedures.

Q: I see a split (double) cluster on my QX600 System. Is my quantification incorrect?

No. A split positive cluster can appear on some assays, but both sub-populations are true positives and quantification is not affected. Set the lower boundary using a wild-type-only well and draw a single threshold that encompasses both sub-clusters as one positive population—do not try to separate them.

Q: I am seeing residual WT amplification in my dark probe channel when running a singleplex reaction.

What should I do?

Consider reducing the input. Keep total DNA input within the validated window per reaction—more input means more sensitivity but more wild-type signal to suppress. Recommend keeping input below 100 ng total DNA per reaction.

Q: How do I set thresholds on a multiplex panel?

Use a positive control to help identify the location of the positive cluster, and set up a wild-type-only control to adjust the threshold and minimize false positives. Threshold each channel so the mutant-positive droplets are clearly separated from background.

Q: How do I design and order assays?

Design and order through the Bio-Rad Mutation Detection Design Engine at bio-rad.com/MutationDesign. FAM/Dark, HEX/Dark, Cy5/Dark and Cy5.5/Dark assays are available in 200, 1,000, and 2,500-reaction sizes; start with the 200-reaction size for assessment and method development.

Need help getting started?

Contact your Bio-Rad Field Application Specialist at bio-rad.com/Specialist

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

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