Accurate and sensitive somatic mutation detection powered by castPCR™ technology

TaqMan® Mutation Detection Assays

- High specificity—mutant allele detection is based on an allele-specific primer, while wild type background is suppressed by the proprietary MGB blocker oligonucleotide
- High sensitivity—assays can detect down to 0.1% mutation in a background of wild type DNA, as demonstrated in spiking experiments
- Wide dynamic range and excellent PCR efficiency—assays demonstrate at least 4 logs of dynamic range and an average PCR efficiency of 100% ± 10%
- Fast, simple workflow—like other TaqMan® Assays, typically requires 3 hours from sample to results, with minimum hands-on time

Cancer research samples often contain rare somatic mutations within a high background of normal wild type DNA. Many mutation detection methods compatible with tumor specimens, including gene sequencing and real-time PCR, have been reported in the literature and are commercially available. However, commercially available kits have various limitations in terms of sensitivity, specificity, cost, workflow, and turnaround time. We have developed sensitive and easy-to-use TaqMan® Mutation Detection Assays to accurately assess mutation status. TaqMan® Mutation Detection Assays were designed based on the novel competitive allele-specific TaqMan® PCR (castPCR™) technology, which combines allele-specific TaqMan® qPCR with allele-specific MGB blocker oligonucleotides that effectively suppress nonspecific amplification from the off-target allele.

Currently, the assay portfolio covers key somatic mutations identified in various cancer genes including, but not limited to, *KRAS*, *BRAF*, *HRAS*, *NRAS*, *EGFR*, *PIK3CA*, *KIT*, *PTEN*, and *TP53* genes, which have been implicated in many types of cancer. These mutations were selected from the comprehensive Sanger COSMIC database for somatic mutations. The target selection was based on frequency of occurrence and input from leading cancer researchers. We will continually add more mutation assays to cover additional cancer gene mutations. For the most updated list of available assays, refer to the TaqMan® Mutation Detection Assay index file at **lifetechnologies.com/castpcr**.

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About the assays

TaqMan® Mutation Detection Assays contain mutant allele assays, which specifically detect one or more mutant alleles, and corresponding gene reference assays, which detect mutation-free regions of the genes in which the target mutations reside (Figure 1). The validated assay set additionally includes corresponding wild type allele assays (not described here; refer to the TaqMan® Mutation Detection Assay protocol for further information).

Two experiment types

Two types of experiments are required for mutation detection analysis:

1. Detection ΔC_t cutoff determination

A mutant allele assay and corresponding gene reference assay are run on three or more wild type gDNA samples that are from the same sample type as the test samples (e.g., gDNA from FFPE tissue samples, Figure 2). ΔC_t values are calculated for each sample run with a mutant allele assay/gene reference assay pair. The average ΔC_t value for all samples is then calculated and is used to derive the detection ΔC_t cutoff value for the mutant allele assay.

2. Mutation detection

A test sample is run with one or more mutant allele assays and a corresponding gene reference assay (Figure 2). The ΔC_t value for the mutant allele assay/gene reference assay pair is calculated, and this value is compared to the previously determined detection ΔC_t cutoff value to determine the sample mutation status.

Optional use of internal positive control (IPC)

You can duplex the IPC reagents with any TaqMan® Mutation Detection Assay to distinguish true target negatives from PCR failure or inhibition (Figure 3).

Figure 1. TaqMan® Mutation Detection Assay types.

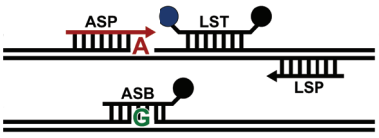
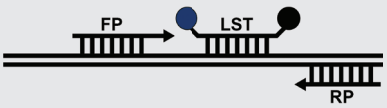
Assay type	Description	Schematic
Mutant allele assay	• Detects specific or multiple mutant alleles • An allele-specific primer detects the mutant allele • An MGB blocker oligonucleotide suppresses the wild type allele	 ASP = Allele-specific primer ASB = Allele-specific blocker (MGB) LST = Locus-specific TaqMan® probe LSP = Locus-specific primer
Gene reference assay	• Detects the gene within which the target mutations reside • A locus-specific pair of forward and reverse primers amplifies a mutation-free region of the target gene	 FP = Forward primer RP = Reverse primer LST = Locus-specific TaqMan® probe

Figure 2. Gene reference and mutant allele assays are run with a genomic DNA sample to determine the mutation status of each target mutation within the cancer gene.

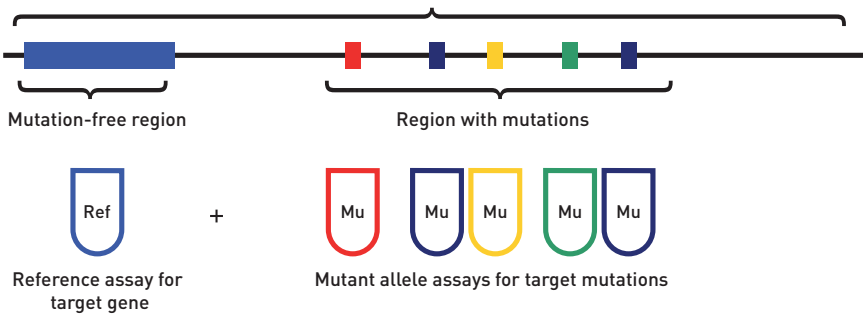
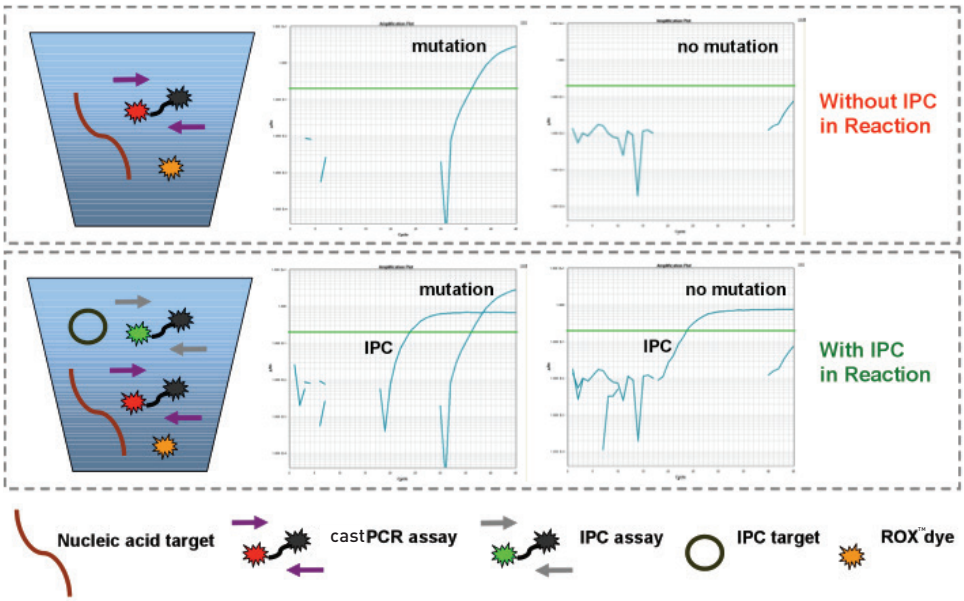


Figure 3. Internal positive controls. The TaqMan® Mutation Detection IPC Reagent Kit is a set of optional internal positive control reagents that can be duplexed with any TaqMan® Mutation Detection Assay to provide a positive PCR control result. The IPC reagents can distinguish a mutation target negative result from a PCR failure result.



Procedure

Purified gDNA, extracted from a sample with an unknown mutation status, is run with one or more mutant allele assays and the corresponding gene reference assay. For each real-time PCR reaction, the gDNA is combined with:

- **A TaqMan® Mutation Detection Assay**—contains two primers and a FAM™ dye–labeled MGB probe to detect a mutant allele or reference gene target. Mutant allele assays also contain an MGB oligonucleotide blocker.
- **TaqMan® Genotyping Master Mix**—contains AmpliTaq Gold® DNA Polymerase UP (Ultra Pure), dNTPs, and buffer
- **(Optional) TaqMan® Mutation Detection IPC Reagent Kit**—contains an internal positive control (IPC) template, two primers, and a VIC® dye–labeled TAMRA™ probe. It can be used to distinguish true target negatives from PCR failure or inhibition.

Reactions are run on a real-time PCR system, using a universal mutation detection thermal cycling protocol. After the run, the real-time PCR system's analysis software determines the C_t values for each TaqMan® Mutation Detection Assay and (optional) IPC reagent reactions. Real-time results export files can be opened in the free Mutation Detector™ Software for post-PCR data analysis. The C_t difference between each mutant allele assay and reference assay is calculated. This ΔC_t value, which represents the quantity of a specific mutant allele detected in a sample, is used to determine sample mutation status by comparison to a previously determined detection ΔC_t cutoff value. You can search for, or download a list of, currently available TaqMan® Mutation Detection Assays at **lifetechnologies.com/castpcr**.

Note: All TaqMan® Mutation Detection Assays have undergone extensive testing to ensure high sensitivity and specificity. The first set of released assays, covering 14 *KRAS*, 29 *EGFR*, and the *BRAF* V600E mutations, underwent additional testing, including determination of: the inherent amplification efficiency difference between mutant allele assays and corresponding reference assays, to enable quantitative analysis of percent mutation in a sample; and assay detection ΔC_t cutoff values using spiked cell line gDNA samples.

Assay performance

Specificity

Mutant allele detection is based on an allele-specific primer, while the wild type allele background is suppressed by the proprietary MGB blocker oligonucleotide. Assays can detect down to 0.1% mutant allele in the presence of a wild type allele background (Figure 4).

High sensitivity

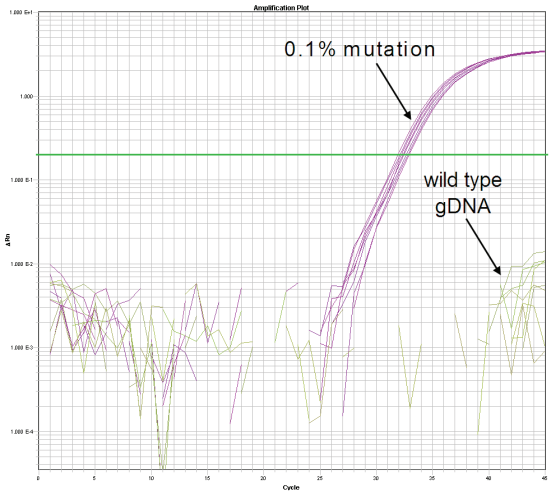
TaqMan® Mutation Detection Assays can detect as few as 1–5 mutant copies in up to one million copies of wild type background. Assay sensitivity is demonstrated using synthetic template spiking experiments (Figure 5 and 6).

Wide dynamic range and excellent PCR efficiency

Assays demonstrate up to 7 logs of dynamic range and an average PCR efficiency of 100% ± 10% (Figure 6).

Figure 4. C_t difference between 0.1% mutation samples and wild type gDNA. For each assay, 0.1% mutant allele samples were obtained by spiking 10 copies of mutant allele synthetic templates into 10,000 copies of cell line wild type gDNA. **(A)** Example of amplification plot for KRAS_522_mu assay on 0.1% mutant allele sample and wild type gDNA. **(B)** There is a significant difference in amplification C_t values between the 0.1% mutant allele sample and wild type gDNA (*P* value < 0.05 for 46 out of 48 assays in the example graph).

A



B

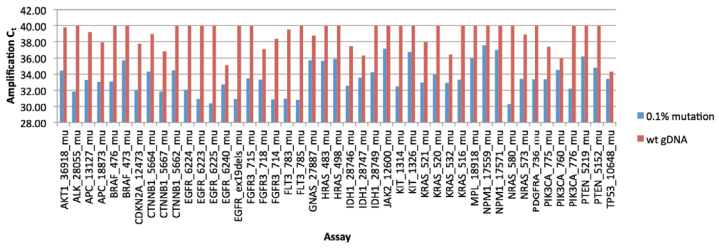


Figure 5. Assay sensitivity and selectivity. For every single assay, the sensitivity and selectivity were analyzed through synthetic template spiking experiments. 10 copies to 10^5 copies of mutant allele synthetic template were spiked into a constant background of 10^5 copies of wild type cell line genomic DNA. For a subset of the assays, 1 copy to 10^6 copies of mutant allele synthetic template were spiked into a constant background of 10^6 copies of wild type allele synthetic template. In the example shown, the BRAF_476_mu assay can detect 1 copy of mutant allele in a background of 10^6 copies of wild type allele.

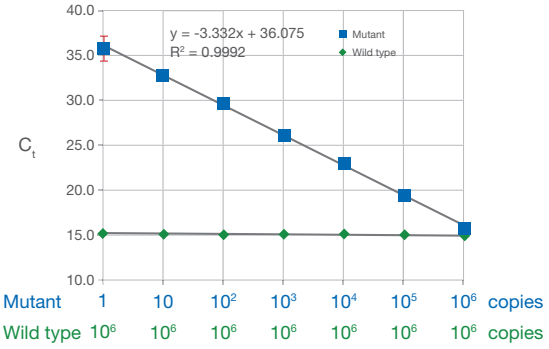
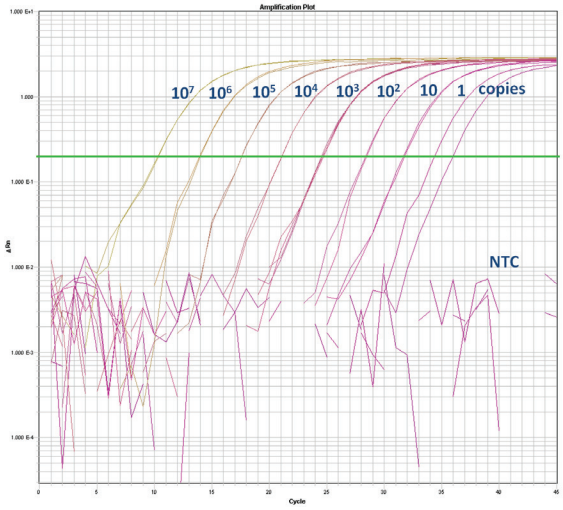


Figure 6. Assay dynamic range. Each assay was tested with 10^5 copies to 10 copies of synthetic template within a constant background of 10^5 copies of wild type genomic DNA. A subset of the assays was tested with 10^7 copies to 1 copy of synthetic template within a constant background of 10^7 copies of wild type allele synthetic template. In the example shown, the KRAS_532_mu assay has 7 logs of dynamic range, with an average PCR efficiency of 100% ± 10%.



Accuracy and reproducibility

Assays demonstrate excellent reproducibility and accurate quantification (Table 1).

Sample type compatibility

The assays can be used with gDNA samples extracted from FFPE tissues, fresh frozen tissues, and cell lines.

Data analysis software

For data analysis, Mutation Detector™ Software allows users to determine the mutation status and quantify the % mutation of their samples from TaqMan® Mutation Detection Assay data collected on the Applied Biosystems® ViiA™ 7, 7900HT, 7500, 7500 Fast, and StepOnePlus™ Real-Time PCR Systems (Table 2).

Table 1. Accuracy and reproducibility. Selected assays were tested in gDNA spiking experiments. In the example shown, G12C mutant cell line gDNA was spiked into wild type cell line gDNA at percentages ranging from 100% to 0.1%. The measured percent mutation was averaged from three experiment runs. The measured percent mutation is highly concordant with the expected percent mutation (R^2 = 0.9997). Accurate and precise quantification (CV < 20%) is obtained among the replicate runs when the target allele copy number is >30.

Copy number, target mutant allele	Expected (%)	Measured (%)	CV (%)
3,000	100.0	100.0	0.0
1,500	50.0	48.9	2.2
750	25.0	23.3	3.8
375	12.5	11.2	7.8
188	6.3	5.7	7.5
90	3.0	2.6	9.0
30	1.0	0.8	17.0
15	0.5	0.4	26.0
3	0.1	0.1	23.0

Table 2. Instrument compatibility.

Applied Biosystems® real-time PCR system	Block module	Software version
StepOnePlus™ system	Fast 96-Well Block Module	StepOne™ Software v2.X
7500 system	Standard 96-Well Block Module	SDS v1.X and v2.X
7500 Fast system	Fast 96-Well Block Module	SDS v1.X and v2.X
7900HT Fast system	Standard 96-Well Block Module, Fast 96-Well Block Module, 384-Well Block Module	SDS v2.X
ViiA™ 7 system	Standard 96-Well Block Module, Fast 96-Well Block Module, 384-Well Block Module	ViiA™ 7 Software v1.X
QuantStudio® 12K Flex system	Standard 96-Well Block Module, Fast 96-Well Block Module, 384-Well Block Module	QuantStudio® Software v1.0

Ordering information

Product	Quantity	Cat. No.
TaqMan® Mutation Detection Assays	150 µL, 10X	4465804
TaqMan® Mutation Detection Reference Assays	150 µL, 10X	4465807
TaqMan® EGFR Exon 19 Deletions Assay	150 µL, 10X	4465805
TaqMan® Mutation Detection IPC Reagent Kit	1 kit	4467538

For more information and full terms of the TaqMan® Assays QPCR Guarantee, go to **lifetechnologies.com/taqmanguarantee**

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