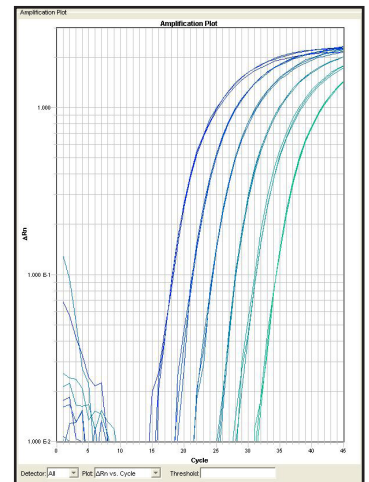


# 5' Nuclease Assays for qPCR

PrimeTime® qPCR Assays offer gold standard performance, design and configuration flexibility, and fast turnaround time you have come to expect from the worldwide leader in custom oligo manufacturing.

The PrimeTime qPCR Assay consists of two primers and a hydrolysis probe with unmatched flexibility. Select from the database of Pre-designed Assays for human, mouse, and rat or design a custom Assay. Both are available with choices for reporter and quencher combinations and primer-to-probe ratios to meet experimental needs. All three components are combined into a single tube and shipped in two to four business days.



## Performance

- Gold standard performance – IDT is authorized by Applied Biosystems to provide 5' nuclease assays.
- PrimeTime qPCR Assays are guaranteed to provide assay efficiency >90% when using Assays generated from IDT's Pre-designed Assay collection or the RealTime Design Tool, a commercially available master mix, and measured over a minimum of four orders of magnitude.
- New Double-Quenched Probes, available only at IDT, contain an internal ZEN quencher and have both lower background and higher signal than traditional dye-quencher combinations.

## Flexibility

- No more black box assays – complete primer and probe sequences are shared upon purchase.
- Choose from five different dye/quencher combinations to best fit any instrument or multiplexing need.
- Select a premixed primer/probe ratio from 1:1 to 4:1 to maximize experimental flexibility and facilitate multiplexing or nontraditional applications.

## Affordability

- Paying for unused reactions? – The PrimeTime Mini allows large cost savings when analyzing high numbers of genes across limited samples and is a perfect tool for discovery or validation.
- The PrimeTime Standard offers twice as many reactions as competitive products at a lower price.
- Screening thousands of samples? – The PrimeTime XL is priced at a fraction of the cost of the competition.

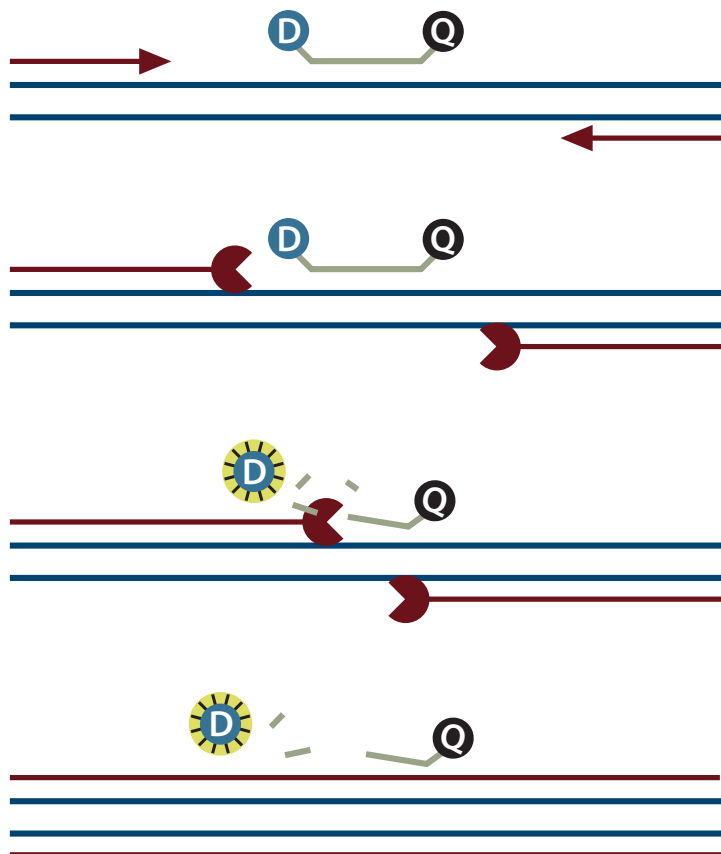
# PrimeTime® Technical Information

UUAACGACCCGALL  
AAGTGAGGGGGGGGAGACTAALL  
TEAACAGTATTGTAAATGCCGTTGGGTA  
FCTTGGCTCATGACCACAGATATAGCGGAAAI  
ATTTTAGTGGTATTATTCCGCTTGGTTTCCATAU  
GGACTTGAAGGACCGTTGGTTGCCCTTGGAGCCTGCL  
GCCGAACATTCCCTCGACTCCAGGAAGATATATCGI  
CAGAAATGATCCAATATCTTACCCTGCCACCCAT  
ACCTGGGTTGCCCGAACCGACAB  
GTCATAGCTATCAATACCTG  
AATGCTAGC

## Introduction to PrimeTime qPCR Assay Technology

PrimeTime qPCR Assays are 5' Nuclease assays, the gold standard for quantitative gene expression studies. The assay consists of a forward primer, a reverse primer, and a hydrolysis probe all delivered in a single tube. The oligonucleotide mixture allows for relative or absolute quantification of a target sequence within a sample. Unlike intercalation dyes, such as SYBR®, the dual-labeled probe allows for improved specificity by increasing fluorescence only when the target sequence is amplified.

During PCR extension, the polymerase cleaves the reporter dye on the 5' end of the hybridized probe, separating the dye from the quenching moiety. The real-time PCR instrumentation detects the fluorescence over a number of cycles allowing for quantitative gene expression measurements.



*The primers and probe hybridize in a sequence-dependent manner to the complementary DNA strand. Because the probe is intact, the fluorophore and quencher are in close proximity and the quencher absorbs fluorescence emitted by the fluor.*

*The polymerase extends from the primers and begins DNA synthesis.*

*The polymerase reaches the probe and the exonuclease activity of the polymerase cleaves the hybridized probe. As a result of cleavage, the fluorophore is separated from the quencher and its fluorescence can be detected.*

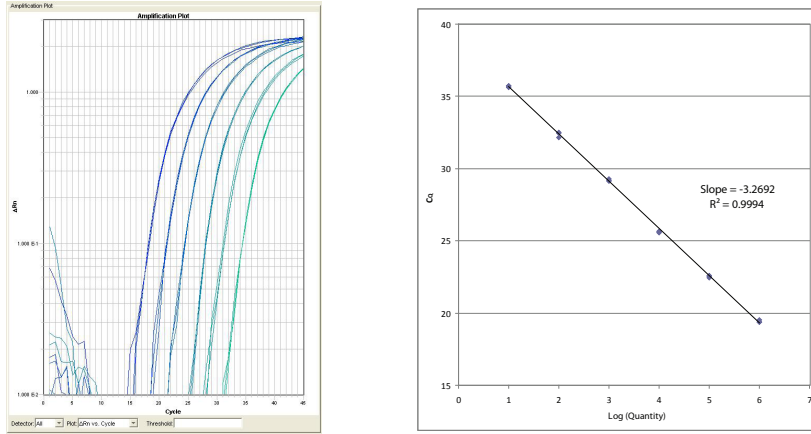
*The fluorescence is detected by the real-time instrument during extension.*

These steps are repeated for each PCR cycle and allow detection of specific products. With intercalation dyes, such as SYBR®, primer dimers and non-specific products will also contribute to fluorescence. In contrast, the 5' Nuclease Assay is specific and fluorescence will only be detected for the DNA sequence to which the probe and primers hybridize.

# PrimeTime qPCR Assay Performance

## Dynamic Range and Sensitivity

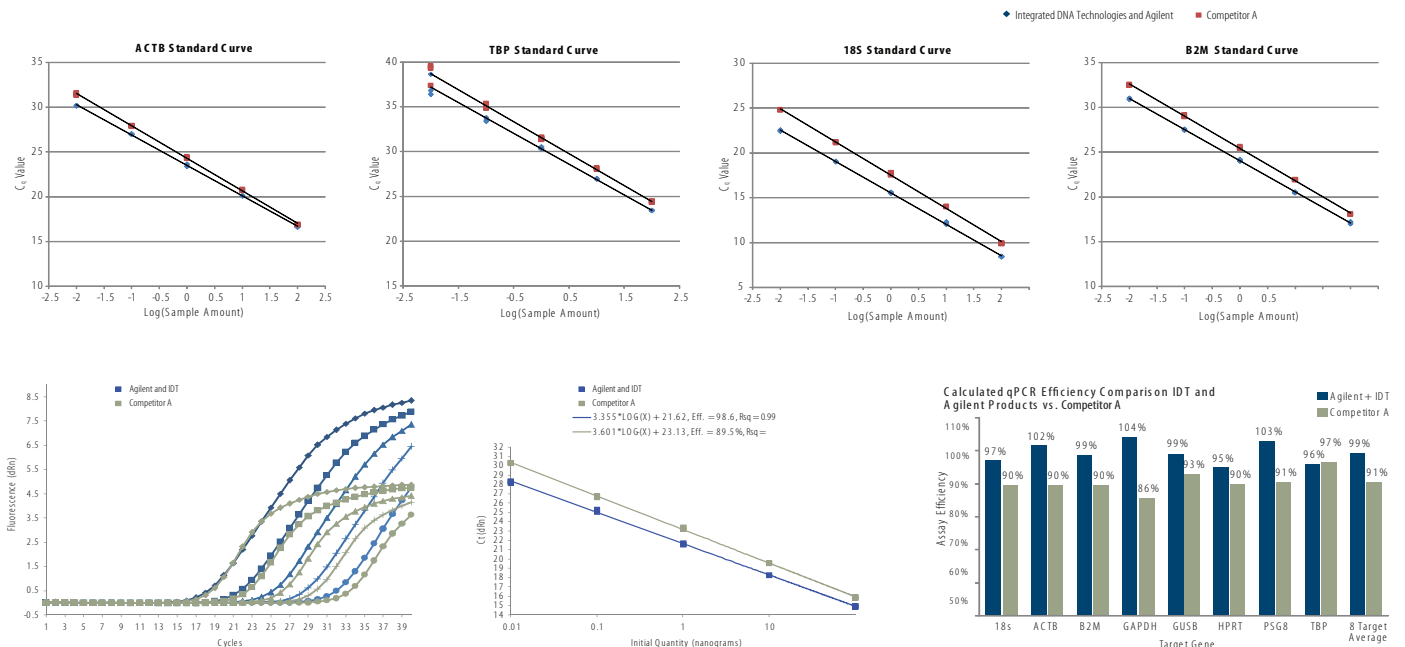
To demonstrate the sensitivity of a PrimeTime qPCR Assay, IDT tested a dilution series over six orders of magnitude down to ten copies per reaction. All dilutions tested produced highly consistent results.



**Figure 1. Dynamic range (6 logs) and 10 copy sensitivity.** The PrimeTime Assay was analyzed by utilizing a plasmid dilution series, and a no template control. The data shown illustrate six logs of dynamic range and assay sensitivity down to 10 copies per reaction. The efficiency of the assay calculated from the standard curve is 102.2% with a correlation coefficient of 0.9994.

## PrimeTime Reliability with Commercially Available Master Mixes

IDT recommends the Brilliant III qPCR Master Mix from Agilent. The combination of the Mx3005P qPCR instrument and Brilliant III qPCR Master Mix from Agilent and the PrimeTime qPCR Assays from IDT provides a fully supported solution, ideal for qPCR validation studies. IDT has also tested other master mixes and found that PrimeTime qPCR Assays demonstrated efficiency close to 100% across many commercially available master mixes.



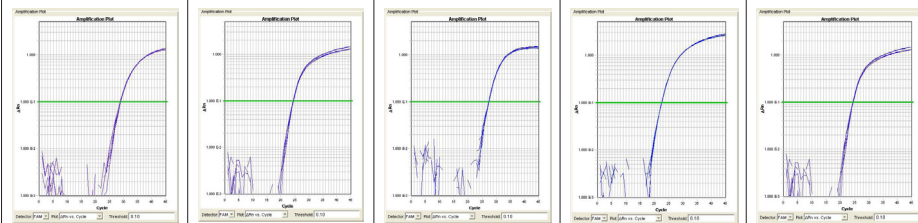
**Figure 2. Comparison of PrimeTime® qPCR Assays with Brilliant II Master Mix, and Competitor A Master Mix and Pre-Designed Assays.** Samples were prepared with AffinityScript cDNA Synthesis Kit from Human Universal Reference RNA from Agilent. A10-fold cDNA dilution series from 100 ng to 0.1 ng was run on the ABI 7900HT system using standard cycling conditions. **Top panel:** Data illustrate Brilliant II and PrimeTime qPCR Assays generate Cq values 1-2 cycles earlier than the competition, particularly at lower input amounts. **Bottom panel:** B2M amplification plots and standard curve are shown along with efficiency comparison for assays across 8 targets. In the B2M assay, the Agilent/IDT assay resulted in 1.5x higher fluorescence and detected each standard 1-2 Cq earlier than Competitor A. The standard curve efficiency values are higher for 7 of 8 Agilent/IDT assays and on average 8% higher. Note: 5 of the 8 Competitor A assays are at or below 90% efficiency.

TaqMan® is a registered trademark of Roche Molecular Systems that is licensed exclusively to Applied Biosystems Inc. for use in certain non-diagnostic fields. QuantiTect® is a registered trademark of QIAGEN group. iTaq™ is a trademark of Bio-Rad Laboratories, Inc. Brilliant® is a registered trademark of Stratagene.

## Reproducibility Across Assay Scales

It is critical that the performance of the Assay remain consistent across scales. IDT tested the Mini, Standard and XL PrimeTime Assays and found reproducibility and precision across all three scales. This attribute allows extension of research from discovery or validation applications to screening applications.

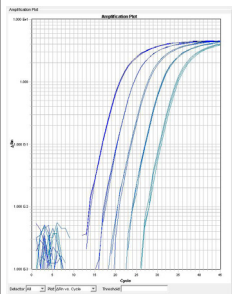
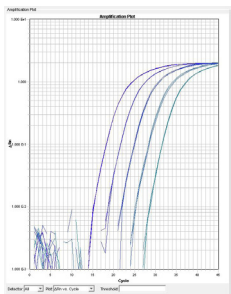
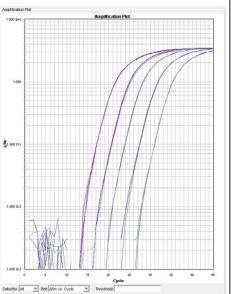
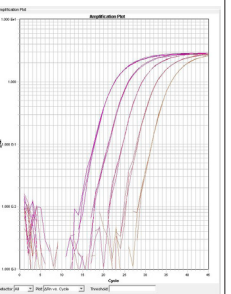
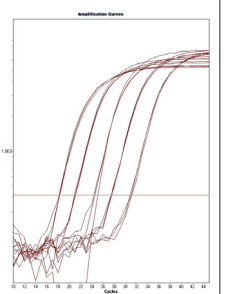
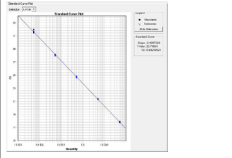
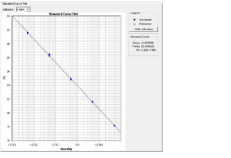
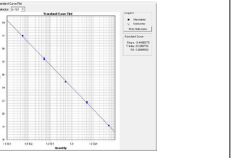
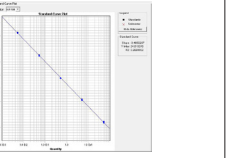
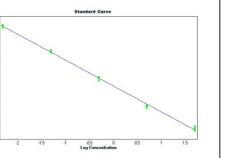
PrimeTime®		Gene ID				
Assay Scale	Replicate	TNFRSF1A	PDK1	JAK2	E2F1	TEC
Mini	1	28.9	24.6	27.5	22.9	29.1
	2	29.1	24.7	27.5	22.9	29.1
	3	28.8	24.6	27.5	22.9	29.1
Standard	1	29.0	24.6	27.3	22.8	29.6
	2	28.9	24.8	27.3	23.0	29.5
	3	28.9	24.6	27.5	22.9	29.6
XL	1	29.0	24.6	27.5	23.0	29.5
	2	28.9	24.6	27.5	23.0	29.4
	3	28.7	24.7	27.6	23.0	29.5



**Figure 3. PrimeTime® qPCR Assays Demonstrate Reproducible Results Across All Three Available Scales.** Five different PrimeTime Mini, Standard, and XL qPCR Assays were manufactured and run in triplicate using the ABI 7900HT Real-time PCR instrument under standard cycling conditions with TaqMan® Gene Expression Master Mix (Applied Biosystems). Each reaction contained 50 ng HeLa cell cDNA prepared with oligo dT and random hexamer primers using SuperScript® II (Invitrogen). The table shows three replicate Cq values with overlaid amplification curves for the five different gene assays at all three scales of PrimeTime qPCR Assays. The data consistently show the reproducibility of PrimeTime qPCR Assays at any scale.

## Dye/Quencher Combinations

To demonstrate the performance of different dye/quencher combinations, IDT tested a dilution series and found robustness in PCR efficiency and R<sup>2</sup> values across all dye/quencher combinations available.

Dye / Quencher Combination	FAM™ / ZEN™ / Iowa Black® FQ	HEX™ / ZEN™ / Iowa Black® FQ	TET™ / ZEN™ / Iowa Black® FQ	FAM™ / TAMRA	Cy5™ / Iowa Black® RQ
Amplification Curve					
Standard Curve					
Efficiency	95%	99%	96%	97%	101%
Coefficient (R <sup>2</sup> )	0.9994	0.9998	0.9997	0.9996	0.9978

**Figure 4. Sample Data Demonstrate Assay Performance with Multiple Dye/Quencher Combinations.** Different Dye/Quencher combinations were tested using a 10-fold cDNA dilution series, from 50 ng to 0.005ng, prepared utilizing AffinityScript QPCR cDNA Synthesis Kit and Universal Human Reference RNA from Agilent. The PCR efficiency and R<sup>2</sup> data illustrate the robustness of PrimeTime® Assays across all dye/quencher combinations and at all tested sample concentrations. All reactions were performed using TaqMan® Gene Expression Master Mix (Applied Biosystems) using standard cycling conditions and, with the exception of Cy5/IBRQ, were run on the AB 7900HT Fast Real-Time PCR System. Cy5/IBRQ was run on the Roche LightCycler 480 II.

## Product Specifications

	Reactions (20 µL)	Estimated Shipping Time	Probe (nmoles)	Primers (nmoles) <sup>1</sup>
<b>PrimeTime Mini</b>	100	2-4 business days	0.5	1
<b>PrimeTime Standard</b>	500	2-4 business days	2.5	2.5-10
<b>PrimeTime XL</b>	2500	2-4 business days	12.5	12.5-50

<sup>1</sup>The primer/probe ratio may be specified by the customer for the PrimeTime Standard and XL Assays. The primer concentration may be 1-4x that of the probe.

- All probes are labeled with a fluorophore at the 5' end and a quencher at the 3' end. Double-Quenched Probes also contain an internal ZEN quencher for reduced-background fluorescence and improved sensitivity over traditional probes.
- The product is delivered lyophilized in a single tube containing pre-mixed primers and probe.
- Each assay is made to order with estimated shipping in 2 to 4 business days from order receipt.
- Each oligo undergoes 100% QC by mass spectrometry. All QC results are provided free of charge to the customer on the IDT website.

### Dye/Quencher Combinations

Dye	Quencher	Mini	Standard	XL
5' FAM	ZEN/3' Iowa Black FQ	•	•	•
5' FAM	3' TAMRA		•	•
5' HEX	ZEN/3' Iowa Black FQ		•	•
5' TET	ZEN/3' Iowa Black FQ		•	•
5' Cy5	3' Iowa Black RQ		•	•

### Instrument Compatibility with Reporter Dyes

	ABI 7000	ABI 7300	ABI 7500	ABI 7900	ABI StepOne	ABI StepOne Plus	BioRad CFX384	BioRad CFX96	BioRad iCycler	BioRad MiniOpticon	BioRad MyIQ2	BioRad MyIQ5	Roche LC480	Stratagene Mx3000P	Stratagene Mx3005P
FAM	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
TET	○	○	○	•	○	○	○	○	○	○	○	○	○	○	○
HEX	○	○	○	○	○	○	•	•	•	•	•	•	•	•	•
Cy5	✘	✘	•	✘	✘	✘	•	•	•	✘	✘	•	•	○	•

•	supplier provided or recommended reporter dyes
○	instrument capable dyes, but may require calibration
✘	instrument incapable of supporting

## Ordering PrimeTime Pre-designed Assays

1. [www.idtdna.com/order/predesignedassay.aspx](http://www.idtdna.com/order/predesignedassay.aspx)
2. Enter the gene name, RefSeq number, or Assay ID.
3. Select the species from mouse, human, or rat.
4. Click on Submit.
5. To sort the results, drag the column that you would like to sort by to the top. Drag more than one column to the top to further refine the search.
6. Select the Assay you would like to order.
7. Click on the Assay Configuration link to select the size (Mini, Standard, or XL), the dye-quencher combination, and the primer-to-probe ratio.
8. Click to Add to Order or Add and Checkout button.

**PrimeTime Pre-designed Assays**

Basic | Batch | Help

Gene Symbol  Species  Human  Rat  Disable Wildcard   
 Mouse

RefSeq  Assay ID

**Results >** [Export](#) | [Clear Results](#) | [Sort By Recommended](#)

Show Filter - Records: 1 - 50 of 50 - Pages: 1

Drag a column header here to group by that column.

<input type="checkbox"/>	Assay ID	Gene Symbol	Species	Ref Seq #	Detects All Variants	Exon Location	Assay Configuration
<input type="checkbox"/>	Hs_PT.42.257169	LOC727930	Human	XR_078989[3]	Yes	3 - 4	<a href="#">Std. FAM/ZEN/IBFO, P:P 2_0</a>
<input type="checkbox"/>	Hs_PT.42.182022	PCDH10	Human	NM_032961[1]	No	1 - 3	<a href="#">Std. FAM/ZEN/IBFO, P:P 2_0</a>
<input type="checkbox"/>	Hs_PT.42.175260	PCDH46	Human	NM_018909[1]	No	1 - 2	<a href="#">Std. FAM/ZEN/IBFO, P:P 2_0</a>
<input type="checkbox"/>	Hs_PT.42.241625	TAB3	Human	NM_152787[1]	Yes	6 - 7	<a href="#">Std. FAM/ZEN/IBFO, P:P 2_0</a>
<input type="checkbox"/>	Hs_PT.42.27267	FAT2	Human	NM_001447[1]	Yes	1 - 2	<a href="#">Std. FAM/ZEN/IBFO, P:P 2_0</a>
<input type="checkbox"/>	Hs_PT.42.107304	CEP170	Human	NM_014812[2]	No	13 - 14	<a href="#">Std. FAM/ZEN/IBFO, P:P 2_0</a>
<input type="checkbox"/>	Hs_PT.42.107317	CEP170	Human	NM_001042404[2]	No	12 - 13	<a href="#">Std. FAM/ZEN/IBFO, P:P 2_0</a>
<input type="checkbox"/>	Hs_PT.42.192230	ZBTB10	Human	NM_001105539[2]	Yes	1 - 2	<a href="#">Std. FAM/ZEN/IBFO, P:P 2_0</a>
<input type="checkbox"/>	Hs_PT.42.192236	ZBTB10	Human	NM_023929[2]	Yes	1 - 2	<a href="#">Std. FAM/ZEN/IBFO, P:P 2_0</a>

## Ordering Custom Assays

The highly flexible RealTime PCR Design Tool allows for complete control of design parameters, such as melting temperature targets, amplicon length, and key master mix ingredient concentrations. Prior to purchase, the tool provides the customer with details about the assay that have been previously hidden by competitors; these include the primer and probe locations, amplicon length, melting temperature and, most importantly, actual primer and probe sequences.

### Custom PrimeTime qPCR Assay order steps:

1. Enter RefSeq accession numbers or gene sequences. Look up an accession number using the RefSeq lookup tool. Click Design Assay to continue.
2. Select the assay location such as the entire coding region or specific exons. Click Design Assay to continue.
3. The tool will design multiple assays across different exons. Select the set you want to order (set 1 is most recommended).
4. Review primer and probe sequences and add the Assay to the order.
5. Select primer-to-probe ratios and dye/quencher combinations.

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,538,848, 5,723,591, 5,876,930, 6,030,787, 6,258,569, and 5,804,375 (claims 1-12 only). The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. Except under separate license rights available from Applied Biosystems, no right under any other patent claim, or to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, or to sublicense, repackaging with other products, or resell in any form, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained from the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA. Certain PrimeTime® Assays may contain one or more proprietary dyes that are sold subject to separate limited licenses. Cy™, is a registered trademark of GE HealthCare, and when applicable, the purchaser obtains a limited non-exclusive sublicense under U.S. Patent Nos. 5,556,959 and 5,808,044 and foreign equivalent patents and other foreign and U.S. counterpart applications to use the Product to perform research. NO OTHER LICENSE IS GRANTED EXPRESSLY, IMPLIEDLY OR BY ESTOPPEL. Use of the Product for commercial purposes is strictly prohibited without written permission from GE HealthCare. Iowa Black® is a registered trademark of IDT. The purchase of this product includes a limited non-exclusive sublicense under U.S. Patent Nos. 7,439,341, 7,476,735 and 7,605,243 and U.S. counterpart applications for use in the purchaser's internal research applications only.

<http://www.idtdna.com/catalog/Usage/Page1.aspx>