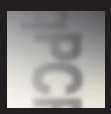
Thermo Scientific Solaris qPCR Gene Expression Assays

Gene-specific qPCR assays with advanced probe technologies



Simplified choice - one assay per target gene is all you need

Accurate and consistent results have never been easier to obtain

Introducing Thermo Scientific Solaris qPCR Assays

One Gene. One qPCR Assay. Simple.

Thermo Scientific Solaris qPCR Gene Expression Assays are pre-designed, gene specific probe and primer pairs that utilize minor groove binder (MGB™) and Superbase technologies to deliver repeatable, sensitive and gene specific quantification.

Through the use of advanced software design and probe technologies, we have adopted a novel approach to designing qPCR probe detection assays. With this approach, we are able to design each assay to a consensus sequence that covers all known splice variants of the target gene. This strategy allows us to recommend **one optimal assay for each gene target**, making it easier than ever to incorporate the benefits of qPCR probe detection assays into your workflow.

Solaris® qPCR Assays

Advancements in qPCR Probe Technology
Resulting in a Simplified User Experience

one assay is all you need

In addition to assay design advancements, Solaris qPCR Gene Expression Assays also offer the following benefits.

- **The sequence information is provided.** Probe and primer sequence information is provided with every assay so you can publish with confidence.
- **The master mix is blue.** Solaris Assays have been developed with our optimized, blue qPCR master mix for the best assay performance and ease-of-use. See your reagents as you pipette, track your progress across multiple wells, and visually assess your set-up.
- **The pack sizes were created to fit your needs.** Solaris Assays are available from small to large pack sizes to accommodate all levels of throughput.
- **The protocol is familiar.** If you currently use qPCR probe assays, Solaris Assays will not require major changes to your protocol or routine.
- **The result is a high performance assay.** You can expect excellent results in terms of repeatability, sensitivity, dynamic range, efficiency and specificity.

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Solaris qPCR Gene Expression Assays

Easy to Choose:
Take the Guesswork out of Selecting an Assay

For the most simplified quantification strategy for your gene target, turn to Solaris qPCR Gene Expression Assays. With one simple search for your gene, you will receive one recommended probe and primer assay for real-time PCR quantification. Since Solaris qPCR Assays are designed to detect all known splice variants of your target gene, one assay is all you need.

What you get

Each pre-designed Solaris qPCR Assay consists of a probe and primer pair specific to your target gene and includes the following:

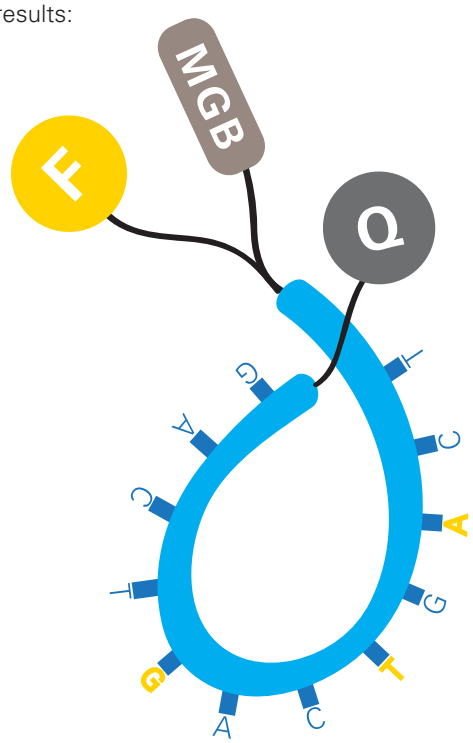
- All assay components at a 20X concentration.
  - o Two primers provided at 800 nM each
  - o One gene specific probe provided at 200 nM
- The sequence information of your gene specific probe and primers
- A high performance Solaris qPCR Gene Expression Master Mix (at a 2X concentration) compatible with your specific qPCR instrument model (ordered separately)

By incorporating advanced chemistries into the design of Solaris qPCR Assays, we can offer gene specific assays that detect even the most challenging design targets, while ensuring every assay will perform optimally under the same thermal cycling conditions. Each assay incorporates the following components to achieve these optimal results:

- Minor Groove Binder (MGB™) moiety
- Superbases (Super G™, Super T™, Super A™)
- FAM reporter
- Eclipse® Dark Quencher

For up-to-date information on predesigned genomes available, visit the web at www.thermo.com/solaris.

More information on these chemistries and how they enhance the performance of Solaris qPCR Assays can be found on page 6.



Schematic representation of the Solaris probe with advanced probe chemistries

The Solaris algorithm incorporates advanced chemistries like MGB and Superbases into the probe design in order to create highly specific assays for even the most difficult design targets.

one assay is all you need

Advancements in qPCR Probe Technology Resulting in a Simplified User Experience

Easy to Use:

Designed, Optimized, and Ready for Quantitative Analysis

Expert Assay Design

The Solaris design algorithm has been developed by experts in the field of bioinformatics and incorporates unique design features and strict selection criteria in order to deliver high performance qPCR assays. Solaris qPCR Assays have been designed using this novel algorithm as well as BLAST scoring to ensure optimal assay specificity.

Universal Thermal Cycling Conditions

As part of the assay design logic, the Solaris algorithm modifies the probe and primer design to ensure assays perform optimally under the same thermal cycling conditions - often referred to as "universal thermal cycling conditions". This is achieved by incorporating an MGB moiety and Superbases into the assays to adjust, and thereby standardize, their melting temperature.

Splice Variant Coverage

To achieve accurate target quantification, the expression levels of all known alternatively spliced transcripts should be measured. Solaris Assays are unique in that they are designed to detect all known splice variants by identifying a "consensus" region, one that is common among all splice variants, and designing the assays within this sequence.

Solaris Master Mixes Give You Optimal Results – and They're Blue!

Optimized Solaris qPCR Master Mixes have been developed to ensure the best possible Solaris Assay performance. In addition to enhanced performance, these optimized reagents offer a unique feature – they're blue. This allows visual confirmation of set-up to eliminate pipetting errors and further enhance the repeatability of your data. Solaris qPCR Master Mixes are compatible with all commonly used real-time instrument platforms (Fig. 1).

Solaris qPCR assays deliver high performance results across qPCR platforms

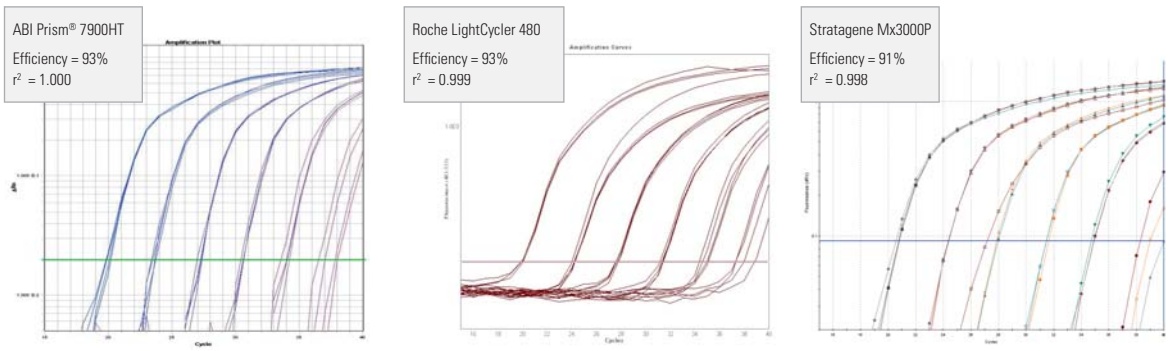


Figure 1: Solaris gives highly reproducible assay results, as judged by the PCR efficiency and r^2 values. PPIB was amplified in quadruplicate from six ten-fold dilutions of cDNA synthesized from 100 ng total RNA on three instruments, ABI 7900HT (384-well format), Roche LightCycler 480 (384-well format) and Stratagene Mx3000P (96-well format). Calculated efficiency and r^2 values are shown on each amplification curve. The average C_q value for the highest amount of input cDNA for the three instruments is 20.5 with standard deviation of +/- 0.6.

Why Choose Solaris qPCR Gene Expression Assays Over Others?

MORE OF WHAT YOU NEED

Compared to other probe detection options, you get more benefits and convenience with Solaris qPCR Assays.

What you get	What this means	Solaris	Supplier A	Supplier Q
Predesigned qPCR assays	Time savings	✓	✓	×
Assay sequence provided	Publish with sequence data	✓	×	✓
>98% Human genome coverage	Complete pathway studies	✓	×	×
Fully optimized master mix	Complete product system for optimal results	✓	✓	×
What you can expect	**What this means**			
Detect all known splice variants of gene	Comprehensive analysis with one assay	✓	×	×
Universal thermal cycling conditions	No preliminary optimization required	✓	✓	×
Low background signal	Improved sensitivity	✓	×	×

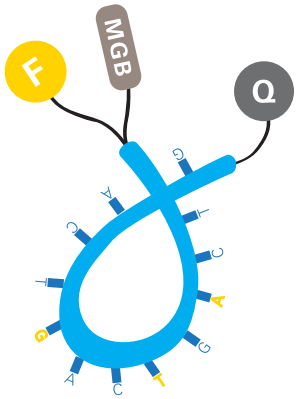
The Most Advanced Probe Configuration and Chemistries

Probe Design Leads to Low Background Signal

In all qPCR probe detection assays, the configuration of the probe plays an important role in the performance of the assay. Consequently, we have designed Solaris qPCR Assays in an ideal configuration of FAM reporter, Eclipse Dark Quencher, and the MGB moiety. This unique arrangement leads to low background fluorescence, independent of temperature over the range of PCR cycling conditions, thus providing a high signal-to-background ratio.

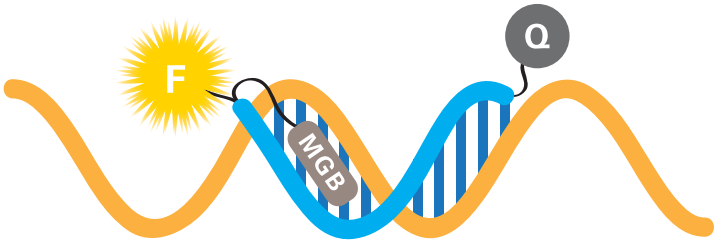
Minor Groove Binder Increases Genome Coverage and Specificity

The proven MGB technology incorporated into Solaris probe design gives a significant advantage over dual-labeled technologies that lack this moiety. The MGB moiety allows for highly specific, shorter probes to be designed while still maintaining the appropriate melting temperature, giving the Solaris algorithm more flexibility or "design space" in which to develop the assay. This increased flexibility is why we can offer predesigned assays for >98% of each genome.



Superbases Enable Assay Design to Difficult Targets

Just as the MGB moiety expands the design capabilities of the algorithm, Superbases perform a similar role. Superbases are chemical derivatives of native nucleotides and can increase the design flexibility of the algorithm by turning difficult probe and primer sequences into viable genomic assays. The use of Superbases allows the algorithm to refine the T_m, reduce secondary structure, improve mismatch discrimination and ultimately help deliver stable assays to even the most difficult design targets.



The Solaris probe fluoresces when bound to it's specific target

The Solaris MGB probe binds to linearized target in the annealing step and is displaced by the advancing DNA polymerase. Once back in its free state, the probe forms a coiled structure where the reporter and quencher are brought into close proximity by the MGB moiety, creating a low background signal.

The Difference is in the Algorithm

The Power of Solaris Design

Our bioinformatics experts have developed a robust algorithm that designs genomic assays based on optimal functionality, specificity, and splice variant coverage. The result is a single recommended, high performance assay for your gene expression experiments. The algorithm incorporates the following design rules.

- Multiple stringent probe and primer parameters for optimal assay performance
- Selective placement of Superbases and use of MGB moiety for increased design flexibility
- Identification of consensus sequence for splice variant coverage (see graphic below)
- Exon junction spanning design when possible to avoid genomic DNA amplification
- BLAST analysis for assay specificity

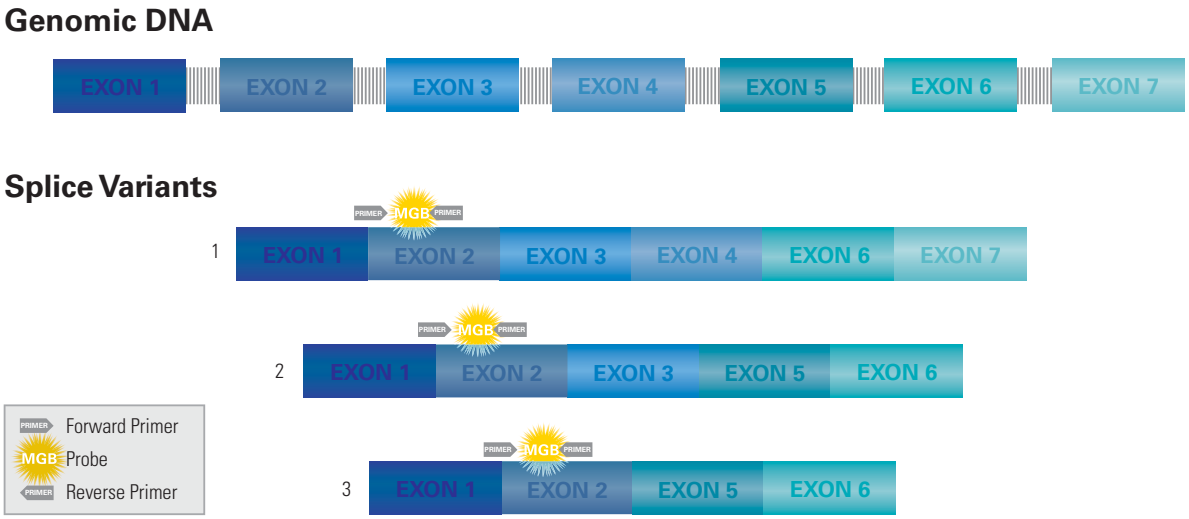
Our powerful design algorithm eliminates the need for preliminary optimization so you can spend less time optimizing your assay and more time deciphering the complex biology of your experiment. The box to the right describes the detailed specifications that our algorithm takes into account for each Solaris qPCR Gene Expression Assay.

Detect all known splice variants of your target gene for comprehensive target analysis.

Solaris qPCR Assays are designed to a consensus sequence among all known splice variants so one assay provides comprehensive results.

Solaris Design Parameters

1. When designing a functional assay, numerous design parameters are applied with high stringency, including: overall GC content, optimal sequence length, melting temperature (T_m), stretches of homogenous nucleotides (i.e. GGGG).
2. The algorithm adjusts the T_m and enables universal cycling conditions by incorporating the MGB moiety and by selective placement of Superbases.
3. When there is more than one splice variant for a target gene, a consensus (or common) sequence is identified, representing design space where assays will detect all known splice variants (See graphic on this page).
4. BLAST analysis is a critical component of any comprehensive qPCR assay design protocol and BLAST analysis has been integrated into the Solaris design algorithm. The algorithm utilizes genomic transcript and pseudogene databases to identify and eliminate sequences that are more likely to lead to erroneous priming and detection (i.e. off-target effects).
5. To mitigate the potential for genomic DNA amplification, the design algorithm, whenever possible, will place one of the assay components (probe or primer) or amplicon over an exon junction boundary.



Why Choose Solaris qPCR Gene Expression Assays Over Others?

High Performance Assays for High Quality Results

Linear Dynamic Range, Sensitivity, and PCR Efficiency

The linear dynamic range of a qPCR assay indicates how many orders of magnitude the assay can amplify consistently and efficiently for the intended target. In robust qPCR assays, the linear dynamic range should span 5-6 $\log_{10}$ dilutions. On the other hand, the sensitivity of a qPCR assay is expressed as the lower limit of detection (LOD). This is the lowest concentration that can consistently be detected in replicate samples. Here, we show high performance of Solaris qPCR Assays in terms of both linear dynamic range and LOD, using synthetic DNA and cDNA templates for amplification (Fig. 2).

Another measure of qPCR assay quality is high reaction efficiency (ideally 95-105%), which indicates a doubling of amplified product with every PCR cycle. Figure 3 illustrates the superb efficiency that can be found across a population of Solaris Assays.

Solaris Assays give high performance results across instrument platforms

	Target	Gene ID	PCR Efficiency (%)	Repeatability (r^2)
ABI 7900HT	RPS1	6222	98	0.996
	CDC45	8318	100	1.000
	ZYX	7791	100	0.998
	RPS27L	51065	100	1.000
	RPLP2	6181	101	1.000
Roche LightCycler 480	C9orf86	55684	97	0.988
	MTHFD2	4522	98	1.000
	BACH1	571	99	0.999
	ACTB	60	100	0.999
	VIM	7431	101	1.000
Mx3000P Stratagene	PPIB	5479	91	0.998
	B2M	567	93	0.999
	POLR2H	5437	93	0.996
	CENPE	1062	98	0.996

Figure 3: Solaris gives efficient, repeatable detection of all gene targets on all commonly used qPCR platforms and targets.

Solaris Assays give high performance and reliable target detection at low input copy number

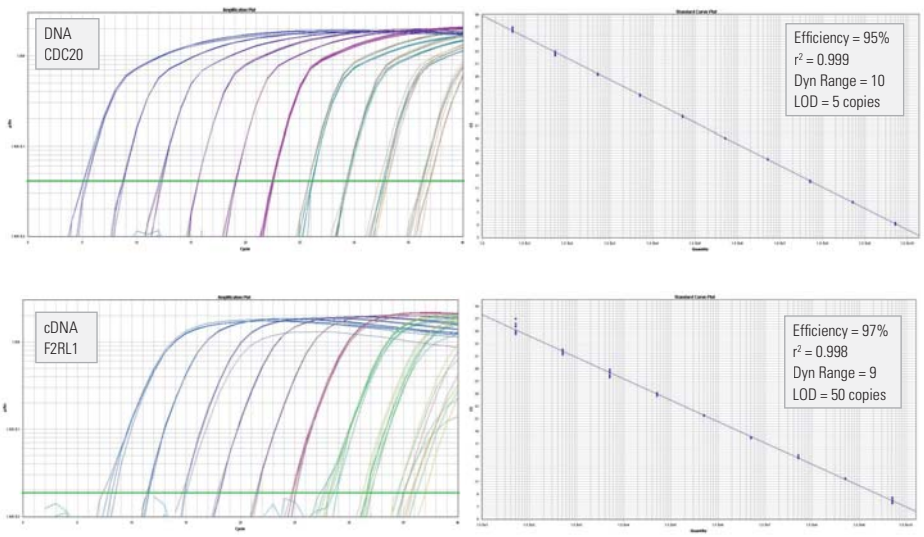


Figure 2: Solaris Assays give reliable detection even at very low input concentrations, as judged by the PCR efficiency and r^2 values. Ten 10-fold dilutions of cDNA synthesized from synRNA amplicon sequence or DNA amplicon sequence was amplified on an ABI 7900HT instrument using Solaris qPCR Gene Expression Assay for F2RL1 or CDC20, respectively. The log-scale amplification curves and standard curves are shown along with the performance of each assay including efficiency, r^2 value, dynamic range out of 10 $\log_{10}$ dilutions and the lower limit of detection.

high performance qPCR assays

Feel Confident that Your Data is Highly Repeatable and Specific

Repeatability & Reproducibility

Repeatability refers to the precision and robustness of the assay when the same samples are repeatedly analyzed in the same assay - in other words, intra-assay variance. Reproducibility refers to inter-assay variance. Both types of variance are minimized with the Solaris system, in part by providing the assay and optimized master mix in 20X and 2X concentrations, respectively, thereby limiting the number of pipetting steps necessary. Excellent reproducibility is shown here in an RNAi knockdown experiment. Detection of the same gene targets and reference gene from a single experiment was performed by two researchers at two different sites. The same level of knockdown was detected for both target genes (Fig. 4).

Specificity

Gene specificity is the amplification of the intended gene target without amplifying genomic DNA (gDNA) or unintentional targets (i.e. off targeting). Solaris Assays are designed to span exon-exon boundaries whenever possible so that amplification of gDNA is avoided. For single exon genes there is potential for gDNA amplification. Similarly, if there is a pseudogene target for an assay that spans an exon-exon junction, contaminating gDNA may be amplified. Anytime these circumstances are true for a given assay, the information is provided to the researcher to allow for complete gDNA removal prior to reverse transcription. Figure 5 demonstrates specificity of Solaris Assays as single amplified bands on an agarose gel.

Solaris Assays are highly reproducible

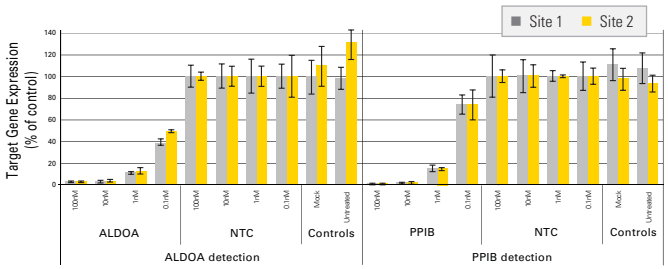


Figure 4: Solaris Assays give consistent results even between different researchers and laboratories. siRNAs targeting ALDOA and PPIB, as well as a Non-targeting Control (NTC), were transfected into HeLa cells at 100, 10, 1 and 0.1 nM final concentrations. Cells were harvested and total RNA isolated 48 hrs post-treatment. cDNA was synthesized using Thermo Scientific Verso cDNA Synthesis Kit. An aliquot of the cDNA was amplified in two geographically separated laboratories using Solaris qPCR Gene Expression Assays for detection of ALDOA, PPIB, and GAPDH on a Roche LightCycler 480 (384-well) platform. Knockdown was calculated using the $\Delta\Delta C_q$ method (normalized to GAPDH reference gene and NTC-treated cells). The same levels of knockdown were demonstrated for both gene targets between both researchers at both sites.

Specificity of Solaris qPCR Gene Expression Assays

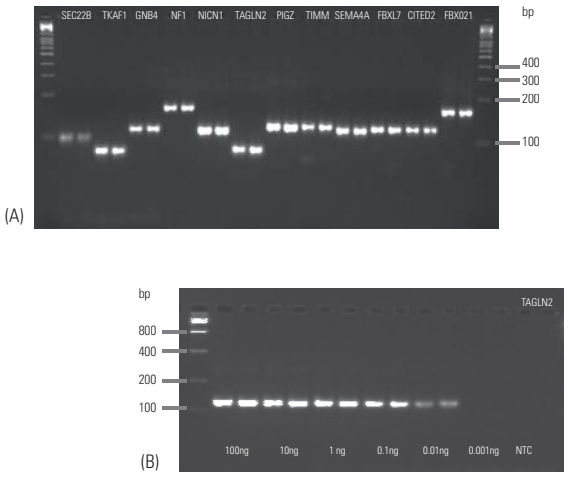


Figure 5: Solaris gives highly accurate results as judged by the specific amplification of the target amplicon. (A) Duplicate wells of qPCR reactions post-amplification (40 cycles; total reaction volume loaded on gel) run on 4% agarose gels (100 ng total RNA input into 20 µL cDNA synthesis reaction). (B) Duplicate wells of six 10-fold cDNA dilutions (100 ng total RNA input into 20 µL cDNA synthesis reaction) and no template control (NTC) run on 4% agarose gel demonstrates specificity of Solaris qPCR Assays.

[1] Bustin,S.A., Benes,V., Garson,J.A., Hellemans,J., Huggett,J., Kubista,M., Mueller,R., Nolan,T., Pfaffl,M.W., Shipley,G.L. et al. (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem., 55, 611–622.

high quality results

Search Your Gene. Find Your Assay. Simple.

Search Your Gene at www.thermo.com/SolarisSearch to Find the Optimal Solaris qPCR Assay.

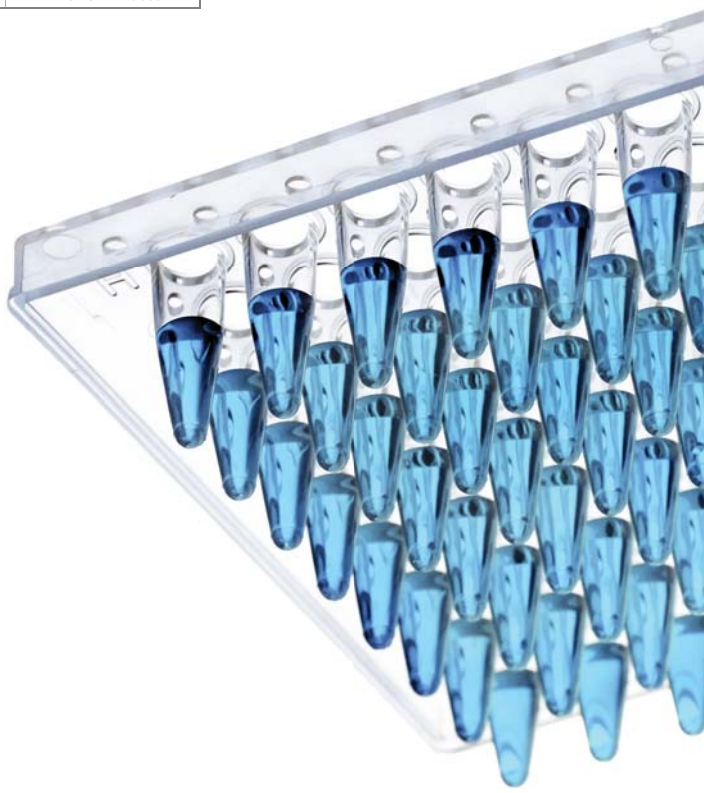
For best results, add optimized reagents at the time of your order.

Solaris qPCR Gene Expression Assays (20X solution)	Part Number	Reactions (25 µL volume)	Fill Volume	Sequence Information Provided
	Gene specific www.thermo.com/SolarisSearch	100	125 µL	✓
		200	250 µL	✓
		400	500 µL	✓
		1000	1250 µL	✓

Solaris qPCR Master Mixes (2X solution)	Part Number	Reactions (25 µL volume)	Fill Volume	Format
	Instrument Specific See table on page 11 or select online	100	1.25 mL	1 x 1.25 mL Tube
		200	2.5 mL	2 x 1.25 mL Tubes
		400	5 mL	4 x 1.25 mL Tubes
		1000	12.5 mL	2 x 6.25 mL Tubes

Verso cDNA Kit	Part Number	Reactions (20 µL volume)
	AB-1453/A	40
	AB-1453/B	100

See back cover for more ordering options.
For up-to-date information on estimated delivery times, please inquire or visit the web.



optimized solaris qPCR master mixes

Solaris qPCR Master Mix Selection Chart

1. Select Your qPCR Cycler

2. Find Your Compatible Mix

	PROBE		
	Mix + ROX Vial	ROX Mix	Low ROX Mix
ABI			
7000		●	
7300		●	
7500			●
7700		●	
7900		●	
7900HT		●	
StepOne™		●	
StepOne™ Plus		●	
Bio-Rad			
iCycler™	●		
MyiQ™	●		
iQ™ 5	●		
Opticon™	●		
Opticon™ 2	●		
Chromo 4™	●		
MiniOpticon	●		
Cepheid			
SmartCycler™	●		
Corbett			
Rotor-Gene™	●		
Eppendorf			
Realplex	●		
Roche			
Lightcycler® 480	●		
Stratagene			
Mx4000®	●		●
Mx3000P®	●		●
Mx3005P®	●		●
Techne			
Quantica®	●		

3. Choose Your Preferred Format

Solaris™ qPCR Gene Expression Master Mix				
Reactions (25 µL volume)	**Format**			
100	1 x 1.25 mL Tube	AB-4350/INT	AB-4351/INT	AB-4352/INT
200	2 x 1.25 mL Tubes	AB-4350/A	AB-4351/A	AB-4352/A
400	4 x 1.25 mL Tubes	AB-4350/B	AB-4351/B	AB-4352/B
1000	2 x 6.25 mL Tubes	AB-4350/C	AB4351/C	AB-4352/C

The complete Solaris detection system requires Solaris qPCR Master Mix for optimal results. Select the high performance mix for your qPCR instrument model using the table here or select a mix online when placing your order.

Need help? Contact our qPCR technical support team

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one assay is all you need

Thermo Scientific Solaris qPCR Gene Expression Assays

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Euro Literature # PB 2009 57

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