Thermo Scientific Solaris qPCR Gene Expression ROX Master Mix

Introduction

Solaris qPCR Master Mix has been developed in conjunction with Solaris qPCR Gene Expression Assays for quantification of cDNA. Optimal qPCR results using probe detection chemistries can be achieved by using this system of qPCR products. These optimized master mixes offer a unique feature – they're blue. Working with blue reagents allows you to see your reagent as you pipette, track your progress across multiple wells, and visually assess your set-up. This visual confirmation further enhances the repeatability of your data. Solaris Master Mixes are available for any real-time instrument platform.

With the exception of probe, primers and template the Solaris qPCR Gene Expression Master Mix contains all the components required for reliable, sensitive and specific qPCR detection. These components include:

- Thermo-Start™ DNA Polymerase – a chemically modified hot-start version of ThermoPrime DNA Polymerase, which prevents non-specific amplification during the reaction set-up. **This enzyme performs optimally with a 95°C for 15 min activation step for complete enzyme activation.**
- Proprietary reaction buffer – a highly optimized buffering system that delivers improved assay performance when used in conjunction with Solaris primer/probe assays. The Solaris Master Mix also contains an inert blue dye to significantly increase the contrast between the reagent and plastic, making verification of master mix dispensing quick, easy and foolproof.
- dNTP – dTTP completely replaces dUTP in the Solaris qPCR Gene Expression Master Mix for maximum amplification efficiency.
- ROX – the master mix includes ROX for normalization of data on ROX-dependent qPCR platforms.

Kit Contents

Vial	*Pack Size (cap color)*			
	INT (100 x 25 µL rxns)	A (200 x 25 µL rxns)	B (400 x 25 µL rxns)	C (1000 x 25 µL rxns)
Solaris qPCR Master Mix (2X)	1 x 1.25 mL (clear)	2 x 1.25 mL (clear)	4 x 1.25 mL (clear)	2 x 6.25 mL (clear)

Information

Cycler Compatibility

Solaris qPCR Gene Expression ROX Master Mix is compatible with all qPCR instruments that require 500 nM ROX for normalization of fluorescent data.

Template Preparation

For maximum sample purity, we recommend extracting total RNA using a spin column method. We also recommend using the Thermo Scientific Verso cDNA Synthesis Kit in the upstream reverse transcription (RT) step, as we have verified that this kit performs well with Solaris reagents.

Storage Conditions

Store at -20°C until ready for use. Solaris qPCR Gene Expression ROX Master Mix is stable for a minimum of 12 months. The reagents can be stored at 4°C for up to 1 month. Avoid repeated freeze thawing. The ROX dye is light sensitive; exposure should be minimized. Shipped on ice internationally and within the US.

Solaris qPCR Gene Expression Assays

Solaris qPCR Gene Expression Master Mixes are designed to be used with predesigned Solaris qPCR Gene Expression Assays for reliable, sensitive and specific detection.

Directions for Use

qPCR Protocol

1. Thaw the reagents on ice, mix the solutions and spin down before use to recover the maximum amount. **Do not vortex the Solaris Master Mix.**
2. Reaction mix preparation:

		96-well Plates	384-well Plates
		Volume	Volume
Reaction Mix	Solaris qPCR Master Mix (2X)	12.5 µL	5 µL
	Solaris Primer/Probe Set (20X)	1.25 µL	0.5 µL
	Water (PCR grade) [1]	Variable	Variable
	cDNA Template (1-250 ng) [2]	1 - 5 µL	1 - 2 µL
	Total volume	25 µL	10 µL

3. Seal plate with optically clear seal and briefly centrifuge to avoid bubbles within the wells, as these may interfere with the fluorescence detection. Always include a no template control (NTC) to assess any potential reagent contamination.
4. Run on thermal cycler.

qPCR Thermal Cycling Program:

	Temp.	Time	Number of cycles
Enzyme activation	95°C	**15 min** [3]	1 cycle
Denaturation	95°C	15 sec	40 cycles
Annealing/Extension [4]	60°C	60 sec	

Notes

1. The volume of the total reaction should be completed up to 10 µL or 25 µL with PCR grade water.
2. The volume of template added can be adjusted as required. For standard templates only 1 µL should be added to reduce carryover of PCR inhibitors. This volume can be increased for low copy number templates.
3. This enzyme performs optimally with a 95°C for 15 min activation step for complete enzyme activation.
4. Fluorescent data to be collected at this step.

10 Minute Enzyme Activation Step

The standard protocol for Solaris qPCR Gene Expression Assays incorporates a 15 minute activation step at 95°C. Solaris Assays also perform well using a 10 minute activation step. We recommend using the protocol below to assess the performance of your assay with a shorter activation step.

	Temp.	Time	Number of cycles
Enzyme activation	95°C	**10 min**	1 cycle
Denaturation	95°C	15 sec	40 cycles
Annealing/Extension	60°C	60 sec	

Fast qPCR Protocol Suggestions

Solaris assays have been developed to give optimal performance under standard thermal cycling conditions. Typically, Solaris assays will also perform well when using a fast qPCR protocol, giving repeatable, specific and sensitive assay results. However, as with any predesigned assay, we recommend that if not following our standard thermal cycling conditions that you check that the performance of your assay (e.g. sensitivity) is not affected. A faster protocol that has worked well in our laboratories, when using Solaris qPCR Gene Expression Master Mix is suggested for your reference below.

	Temp.	Time	Number of cycles
Enzyme activation	95°C	**10 min**	1 cycle
Denaturation	95°C	5 sec	40 cycles
Annealing/Extension	60°C	20 sec	

Shipping and Storage Conditions

Stability tests show that Solaris qPCR Gene Expression Assays are stable for several weeks at room temperature. Solaris Assays are shipped on wet ice to provide additional assurance during transit.

For long-term storage we recommend that Solaris qPCR Assays and Master Mixes are stored at -20°C. We also suggest aliquoting into convenient working stock volumes to avoid repeated freeze-thaws of the product.

Following dilution of Solaris qPCR reagents to a final 1X working concentration we recommend running your experiment immediately. However, data generated in our laboratories demonstrate that assays are stable at a 1X concentration for at least 24 hours, providing you with confidence that assay performance will be unaffected within this time-frame (please refer to our technical note entitled "Stability in the Laboratory").

Ordering Information

AB-4351/INT	Solaris qPCR Gene Expression ROX Master Mix (Intro Pack Size)	100 x 25 µL rxns
AB-4351/A	Solaris qPCR Gene Expression ROX Master Mix	200 x 25 µL rxns
AB-4351/B	Solaris qPCR Gene Expression ROX Master Mix	400 x 25 µL rxns
AB-4351/C	Solaris qPCR Gene Expression ROX Master Mix	1000 x 25 µL rxns

Related Products

Cat No.	Description	Quantity
AX-XXXXXX-XX-XXXX*	Solaris qPCR Gene Expression Assays	100 rxns (1 x 125 µL) 200 rxns (2 x 125 µL) 400 rxns (4 x 125 µL) or 1000 rxns (10 x 125 µL)
AB-1453/A AB-1453/B	Verso cDNA Synthesis Kit	40 x 20 µL rxns 100 x 20 µL rxns
AB-0800/W	ABgene 96-Well PCR Plate (Skirted, Low Profile, White) **	25 plates
AB-2150/W	ABgene Diamond Ultra 384-Well PCR Plate, White **	50 plates
AB-1170	ABsolute™ qPCR Adhesive Seal	50 sheets

* Catalog number is product-specific. Please refer to www.thermo.com/solaris to select the appropriate assay.

** For cycler compatibility and color choices, see our latest catalog or visit www.thermo.com/pcrplastics

Troubleshooting

For technical information or troubleshooting contact Thermo Scientific Genomics Tech Support:

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North America (US, Canada, Central/South America)	Techservice.genomics@thermofisher.com	+1 (800) 235-9880
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