REAL-TIME qPCR

SAMPLE TEST GUIDE

ANASPEC EGT GROUP

Eurogentec
Experience true partnership

Introduction

The following recommendations should be considered before beginning the comparison test.

Always refer to the kit's recommended protocol as starting point for the comparison test (Eurogentec kits always contain a TDS in the kit box).

THIS BOOKLET OFFERS tips and guidance to help successfully compare a Eurogentec Real-Time qPCR kit with the one that you are currently using. Reaching maximum sensitivity and specificity for a qPCR assay often requires some optimisation, especially at the primer/probe concentration level, and, to a lesser extent, at the cycling parameters level.

The kit type also greatly impacts qPCR results and should be selected carefully based on your Real-Time thermocycler model (FAST vs. non-FAST), and experimental constraints like, for example, the nature of the samples, the need for a carry-over prevention step, the ease of use of the kit, etc...

For all these reasons, it is important, when comparing kits, to follow strict rules that will ensure accurate comparison data, because optimal parameters for one kit may not match those of another kit!

1. **Compare the components** of the kits before starting your experiment

Review the component list of each kit before running the comparative assay. This will help to fairly and accurately assess the side-by-side performance of each kit. The following components can vary from kit to kit:

Taq polymerase

If the kits that are compared contain different types of *Taq*, the comparison test should be done on separate plates and the cycling protocol should be adapted with the proper activation time as recommended by the supplier.

Most kits (like those offered by Eurogentec) contain a chemically modified hotstart *Taq* polymerase, activated within 3 to 10 min at 95 °C. This avoids the amplification of aspecific products during the reaction set-up. However, some competitor kits use other HotStart approches (*eg.* antibody, affibody,...) usually activated within 20 sec to 3 min at 95 °C which have a tendency to be more leaky. **It is of utmost importance that the polymerase is fully activated!** All EGT FAST and MESA kits require 5 min activation, Takyon™ and QGS kits need 3 min activation, while other EGT kits require 10 min activation.

'Fast' vs. 'Regular' protocols

Regular MasterMixes are not suited for FAST cycling conditions (unless stated differently). Always compare kits under recommended cycling conditions. A "regular" kit will probably compare poorly to a FAST kit in FAST cycling conditions, while the opposite is not true.

Never use FAST-like cycling conditions (i.e. reduced step duration) on a "regular" cycler that is not suited for FAST cycling. All Eurogentec FAST & Takyon™ kits perform extremely well in regular cycling conditions.

The kits that are to be compared should ideally both contain either a dNTP/dUTP blend or 100 % dNTP.

100 % dNTP mix vs. dNTP/dUTP mix

The *Taq* polymerase will preferentially incorporate naturally occurring bases such as dTTP rather than dUTP. Therefore, a 100 % dNTP kit will give more sensitive results than a kit containing a dNTP/dUTP blend, depending on the sequence of the template (sequences rich in thymine will benefit more from a 100 % dNTP kit). The presence of dUTP within the mix is mandatory to prevent carry-over contamination using UNG (see next §).

If only one of the two kits that are compared contains UNG, the test should be performed by adding UNG (to the kit that doesn't contain UNG) or without activating the UNG at all.

Kit containing UNG vs. one that does not contain UNG

Uracyl-N-Glycosylase is used for carryover prevention. The enzyme catalyzes the hydrolysis of single-stranded or duplex DNA containing uracil. Usually UNG is activated after 2 minutes at 50°C before the normal steps of a cycling protocol.

Uracil-N-glycosylase

#RXN	Volume/RXN	Reference
600 reactions (300 U)	25 µl	RT-0610-03
3000 reactions (1500 U)	25 µl	RT-0610-15

If the competitor kit uses a lower concentration of $MgCl_2$, the concentration of primers should be decreased when using the Eurogentec kit. To obtain optimal results, a primer titration is recommended when testing a new kit (see Section 4).

Alternatively, differencies in $MgCl_2$ concentrations can be compensated by optimizing the annealing temperature of the cycling protocol (see section 5)

$MgCl_2$ concentration

Eurogentec MasterMixes contain a final concentration of either 2.5 mM (Takyon™ SYBR® Mix) 4.0 mM (MESA kits), 5.0 mM, or 5.5 mM (Takyon™ Probe Kits) $MgCl_2$. Differences in $MgCl_2$ concentrations between two kits should be taken into account, particularly when performing a SYBR® Green I assay. Indeed, Mg^{2+} concentration greatly influences the Tm of the primers, hence the sensitivity and specificity of the results.

Passive reference

Some thermal cyclers require a passive reference to normalize the results.

On an ABI Prism® 5700, 7000, 7300, 7700, 7900 HT, StepOne/Plus or some MasterCycler®, a kit containing ROX passive reference should be used. A kit containing a lower concentration of ROX passive reference should be used on an ABI Prism® 7500 and on ViiA™7. This is also an option on the Mx4000®, Mx3000P® and Mx3005P®.

If no ROX normalization is needed, then a Eurogentec NO ROX kit should be used (LC480, iCycler iQ®, iQ™5, MyiQ, CFX96, RotorGene® 2000/3000/6000/Q, SmartCycler®, Opticon®, MiniOpticon, Chromo4, Quantica®, LightCycler® Nano system and optionally on Mx4000®, Mx3000P® , Mx3005P® and MasterCycler® v.2).

On an iCycler iQ®, iQ™5 or MyiQ, a kit containing fluorescein must be used when performing a SYBR® Green I assay.

When comparing two different kits, always check that both kits contain the reference dye needed.

Flurescein additive

Volume	Reference
1 ml	RT-FLUO-ADD

ROX passive reference

Volume	Reference
300 µl	RT-PARE-03

2. **Clean working** practices

- Perform the reaction set-up in a separate room from the one used for DNA extraction or PCR product analysis
- Use pipettes with sterile filter tips or positive displacement pipettes
- Use optically clear caps or sealers to obtain maximum signal
- Check the proper closure of the caps or sealers
- Wear disposable plastic gloves to avoid contamination and fingerprints on the optical surface of the caps or sealing film that can interfere with signal detection.

3. **Starting** material

Perform the test using the same DNA/cDNA/RNA from the same extraction to avoid any variations deriving from sample preparation.

4. **Perform a primer** or primer/probe titration

Optimal results may require titration of primers and probes. Use the same primers and probes from the same synthesis batches. Use validated/recommended conditions for the competitor's kit and use the matrix below to find the optimal primers/probes concentration for the EUROGENTEC kit. Select the primer pair that yields the highest $\triangle$Rn and the lowest Cq value.

SYBR® Green I assay Recommended primer optimisation matrix

	Forward Primer		
Reverse Primer	50 nM	100 nM	300 nM
50 nM	50 / 50	50 / 100	50 / 300
100 nM	100 / 50	100 / 100	100 / 300
300 nM	300 / 50	300 / 100	300 / 300

Probe assay Recommended primer optimisation matrix

	Forward Primer		
Reverse Primer	50 nM	300 nM	900 nM
50 nM	50 / 50	50 / 300	50 / 900
300 nM	300 / 50	300 / 300	300 / 900
900 nM	900 / 50	900 / 300	900 / 900

Probe assay Recommended primer optimisation matrix

	Probe		
Opt. primers conc.*	50 nM	100 nM	250 nM

*Optimised primers concentration as determined above

5. **Annealing** temperature

Differences in $MgCl_2$ concentrations, or in MasterMixes formulations, can have a huge impact on primer Tm, hence on sensitivity and specificity of the assay. It is possible to compensate for such differences by optimizing the annealing temperature of the cycling protocol.

This procedure can be significantly simplified by using a gradient cycler, which allows several temperatures to be tested in a single run. We recommend to test annealing temperatures ranging from -4°C to +4°C vs. estimated primer Tm.

6. High quality plastics

Always use high quality plastics for your qPCR experiments. High quality plastics will allow you to achieve maximum reproducibility and sensitivity, higher signal-to-noise ratios and $\triangle$Rn.
Always perform comparisons on the same type of plate.
High quality plastics are available from Eurogentec. There are specific plates for each machine and application on the market. Also all Eurogentec plates use Virgin Plastic, meaning that the plates are more rigid, and hence more resistant to bending under temperatures associated with cycling.
See http://www.eurogentec.com/products/qpcr-plastics.html.

7. Plate preparation

Comparison of the Competitor and EUROGENTEC kits should be performed on the same plate, using the same cycling conditions, unless otherwise advised (see below).

- If the type of *Taq* used in the Competitor kit requires a different activation time, Eurogentec recommends that (a) the activation time recommended by the supplier should be used in each cycling protocol, and (b) the comparison test should be run on two separate plates and using the same well positions on each plate. The background should be calculated identically for the two separate cycling protocols (between cycles 0 and 12 for example).
- Prepare a reaction mix volume 10% greater than required for the total number of reactions to be performed. This volume increase will help to minimize errors that are often observed when pipetting smaller volumes. First add the DNA template to the side of each well followed by the appropriate volume of reaction mix. Before starting the cycling protocol, centrifuge the plates gently to properly mix the DNA template/reaction mix and to remove any bubbles.
- See plate set-up diagrams for recommendations of sample placement
- Use exactly the same template (DNA/cDNA/RNA), from the same extraction. Use the same batch of primers and probe. Use the same plastics (if separate plates are used).
- Include a dilution series of known concentrations to compare PCR efficiencies of both kits.
- Include a primer optimisation series to ensure that only optimal results obtained with the new kit are compared with the existing one.
- Always include a No Template Control (NTC) for each kit. The No Template Control should be located on the top or bottom row to avoid going over the well when pipetting and also away from the highest DNA concentration to avoid cross contamination.
- Perform duplicates or even triplicates for each dilution.

Simplified plate set-up for comparison of two different kits when using SYBR® Green I

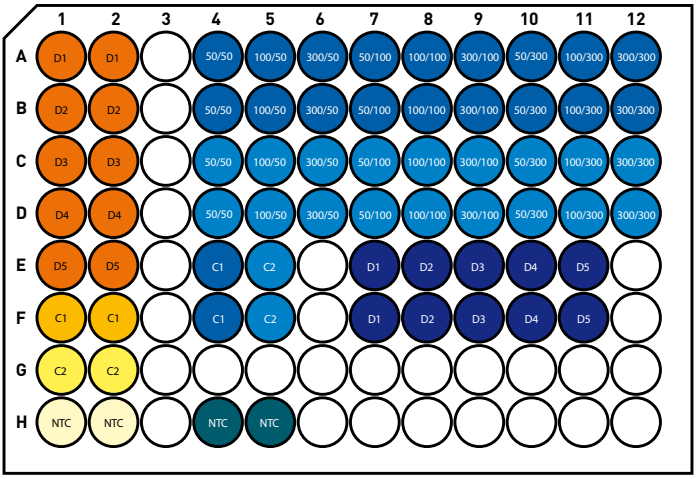
Competitor kit
- Dilution series (D) (see section 7)
- Sample 1
- Sample 2

Eurogentec kit
- Dilution series (D) (see section 7)
- Sample 1
- Sample 2 (if 25 µl final reaction volume is used)

C: Standard conditions

NTC: Non Template Control

100/300:
100 nM final concentration for forward primer and 300 nM final concentration for reverse primer



Simplified plate set-up for comparison of two different kits when using probes

Competitor kit
- Dilution series (D) (see section 7)
- Sample 1

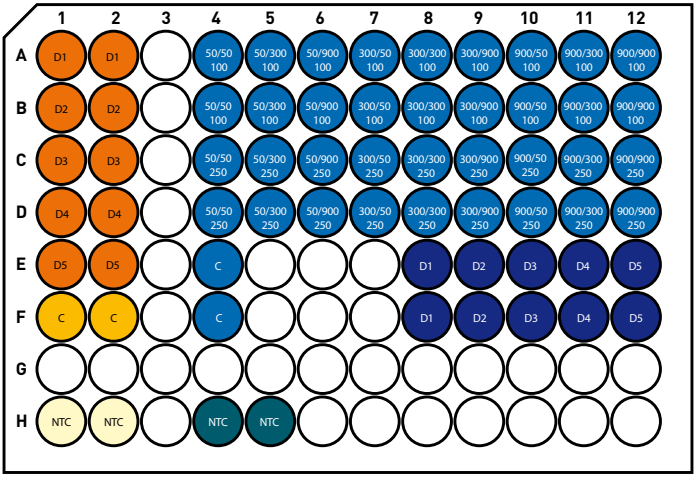
Eurogentec kit
- Dilution series (D) (see section 7)
- Sample 1

C: Standard conditions

NTC: Non Template Control

100/300:
100 nM final concentration for forward primer and 300 nM final concentration for reverse primer

100: 100 nM final concentration for probe



8. **General** recommendations

Perform a standard curve

A standard curve is a dilution series of known concentrations and is a quick way to check for PCR efficiency. The slope of the log-linear phase reports the efficiency of the amplification reaction (a 100 % efficiency corresponds to a slope of –3.32).

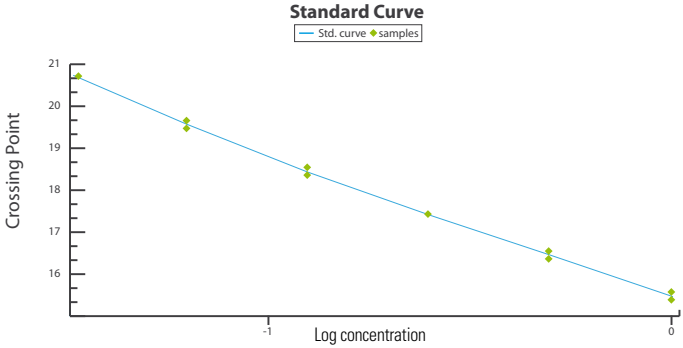


Figure A:
Example of a 2 times-serial diluted cDNA standard curve. Detection using SYBR® Green I on a LC480 thermocycler.

The amplification efficiency of a primer pair should always be as near as possible to 100%. Likewise, efficiencies of multiplexed primer pairs should be nearly identical to ensure valid relative quantifications.

When a high efficiency cannot be reached under rapidly optimised cycling conditions, it is recommended to design a new primer pair, avoiding a complicated, time-consuming optimisation phase.

Note that some qPCR data analysis software do correct for variations in PCR efficiencies between compared assays.

Perform a meltcurve when running a SYBR® Green I assay

Melt curve analysis will give information on the specificity of your assay, including primer-dimer formation, amplification of aspecific products, etc.

A specific amplification will result in a unique, well defined peak.

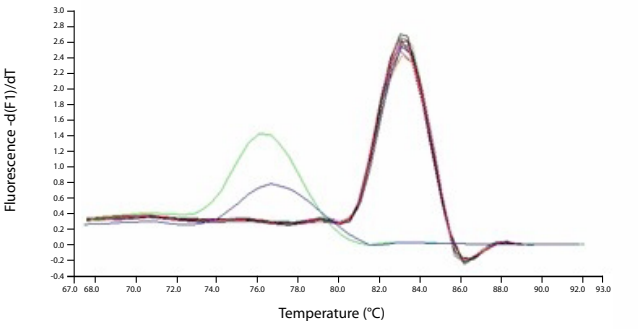


Figure B:
Meltcurve with primers dimers (corresponding to the first peak at around 76 °C).

Use different controls

- Always include a No Template Control (NTC) containing all the qPCR components with the exception of the DNA template. If a product is amplified, it indicates that one or more of the qPCR reagents are contaminated with DNA or that your reaction mix suffers from the formation of primer-dimers.
- Always include internal controls (2 min.) validated within your experiment context.
- Real-Time PCR assays are prone to inhibition by various substances found in many samples (clinical, soil, plant...). Carryover of reagents used for the isolation of nucleic acids can also inhibit amplification reactions. Other causes of false-negative results include target nucleic acid degradation, sample processing errors and thermal cycler malfunction. Use our Universal Exogenous qPCR Positive Control for TaqMan® Assays to distinguish true target negatives from false negatives.
- For a one step RT (reverse transcriptase) qPCR experiment, use a negative RT control. The mix should contain all the reaction components except the reverse transcriptase. If PCR amplification is observed, DNA contamination can be assumed.

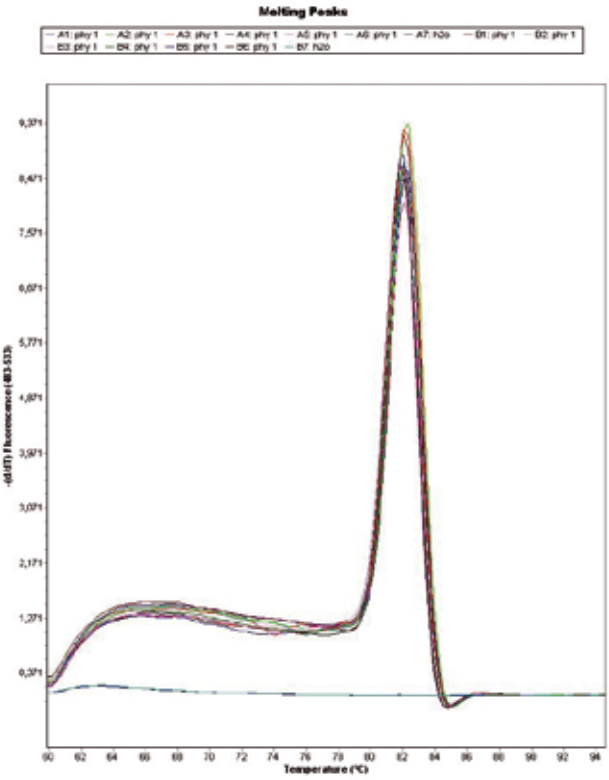


Figure C: Clean meltcurve (without any primers dimers).

9. **Analysis of** the results

Make sure that the results of both kits are displayed equally. The thresholds should be placed at the same level and all graphs should display the same scales. The performance of the kit will be judged on what is important for you. Some important factors to consider include:

Cq Value (quantification cycle)

Usually the Cq value is the most important factor to consider when performing a comparative qPCR assay. The Cq will determine the sensitivity of your assay for each kit whitin your experimental context.

Reproducibility

Reproducibility between replicates and separate assays should always be as high as possible (Δ0.5 Cq max.).
When working with small volumes, make sure that the pipetting step is made under appropriate conditions, with an adequate instrument. Template pipetting errors are the major cause of variability.

PCR efficiency

The PCR efficiency of the assay should be as close as possible to 100% in order to perform a correct quantification.

ΔRn

ΔRn plays an important role in SNP detection: a higher ΔRn is the direct result of better separation between both SNP variants. The level of ROX passive reference in the kit heavily influences ΔRn values. Kits with very low ROX levels are likely to result in higher ΔRn values but will provide less protection against sample variations (due to pipetting errors).

For more information, please contact our scientific support:

EU
scientific.support@eurogentec.com
Tel: +32 (0)4 372 76 65
Toll free number: 00 800 666 00 123

US
tech@anaspec.com
Tel: +1 510-791-9560
Toll free number: +1-800-452-5530

Discover
your new qPCR Solution!

Takyon

Amplify realtime and beyond

see pages 12-15

Takyon™ qPCR kits

Ensure sensitivity and fast delivery of accurate and reproducible results!

www.eurogentec.com/qpcr-takyon.html ♦ info@eurogentec.com

Assay Dispensing Service

Eurogentec
Experience true partnership

Secure your in-house qPCR **Dx assays**

Improve

- Quality Standards
- Cost Efficiency vs CE
- Assay Precision

Simplify

- ISO 15189 Accreditation
- Reagents QC
- Set Up

Testimonials

Pr. Rémi CHARREL
Laboratory of Bacteriology APHM - Timone Hospital, France

The best solution to simplify accreditation of in-house PCR & qPCR assays, combining optimal profitability, efficiency and ease-of-use.

A happy Customer
Laboratory of Microbiology - London Hospital, UK

I have about five lots of 12 strips to get through and there has been no diminution in CT value even though they expired...

My colleagues have been looking on in envy and are keen to experience the joys of pre-aliquoted strips. Recently we had a meeting to discuss what we might need if we used pre-aliquoted strips for all our current routine qPCR assays.

www.eurogentec.com/dispensing.html • dispensing@eurogentec.com

Eurogentec is part of Kaneka Corp.

Table 1 - Kits with ROX passive reference - ABI Prism® 5700 • ABI Prism® 7000 • ABI Prism® 7300

Product name		# RXNs per single kit 20 or 25µL[1]	Reference		
			Single kit	5-pack	10-pack
SYBR® Assays					
MESA GREEN qPCR MasterMix Plus for SYBR® Assay	7.5 ml	600	RT-SY2X-03+WOU	05-SY2X-03+WOU	10-SY2X-03+WOU
	15 ml	1200	RT-SY2X-06+WOU	05-SY2X-06+WOU	10-SY2X-06+WOU
	50 ml	4000	RT-SY2X-20+WOU	05-SY2X-20+WOU	10-SY2X-20+WOU
MESA GREEN qPCR MasterMix Plus for SYBR® Assay dTTP	7.5 ml	600	RT-SY2X-03+WOUN	05-SY2X-03+WOUN	10-SY2X-03+WOUN
MESA BLUE qPCR MasterMix Plus for SYBR® Assay	7.5 ml	600	RT-SY2X-03+WOUB	05-SY2X-03+WOUB	10-SY2X-03+WOUB
NEW Takyon™ ROX SYBR® MasterMix blue dTTP	7.5 ml	750	UF-RSMT-B0701	UF-RSMT-B0705	UF-RSMT-B0710
qPCR Core kit for SYBR® Green I		1000	RT-SN10-05	05-SN10-05	10-SN10-05
qPCR Core kit for SYBR® Green I dTTP		1000	RT-SN10-05NU	05-SN10-05NU	10-SN10-05NU
NEW Takyon Rox SYBR Core Kit Blue dTTP		1250	UF-RSCT-B0201	UF-RSCT-B0205	UF-RSCT-B0210
qPCR Core kit for SYBR® Green I only dUTP		1000	RT-SN10-050U	05-SN10-050U	10-SN10-050U
qRT-PCR SYBR® Assays	**All above kits may be combined with our reverse transcriptase core kit RT-RTCK-03**				
One step MESA GREEN qRT-PCR MasterMix for SYBR® Assay	7.5 ml	600	RT-SYRT-032X	05-SYRT-032X	10-SYRT-032X
Probe Assays					
qPCR MasterMix (5 x 1.5 ml)		600	RT-QP2X-03	05-QP2X-03	10-QP2X-03
qPCR MasterMix Plus	7.5 ml	600	RT-QP2X-03-075+	05-QP2X-03-075+	10-QP2X-03-075+
	15 ml	1200	RT-QP2X-03-15+	05-QP2X-03-15+	10-QP2X-03-15+
	50 ml	4000	RT-QP2X-03-50+	05-QP2X-03-50+	10-QP2X-03-50+
qPCR MasterMix Plus w/o UNG	7.5 ml	600	RT-QP2X-03WOU+	05-QP2X-03WOU+	10-QP2X-03WOU+
qPCR MasterMix Plus dTTP	7.5 ml	600	RT-QP2X-03+WOUN	05-QP2X-03+WOUN	10-QP2X-03+WOUN
NEW Takyon™ ROX Probe MasterMix UNG	7.5 ml	750	UF-RPMU-C0701	UF-RPMU-C0705	UF-RPMU-C0710
NEW Takyon™ ROX Probe MasterMix dTTP BLUE	7.5 ml	750	UF-RPMT-B0701	UF-RPMT-B0705	UF-RPMT-B0710
Takyon™ ROX Probe MasterMix dTTP	7.5 ml	750	UF-RPMT-C0701	UF-RPMT-C0705	UF-RPMT-C0710
qPCR MasterMix Plus QGS	7.5 ml	600	RT-QP2X-03+QGS	05-QP2X-03+QGS	10-QP2X-03+QGS
NEW qPCR Core kit		1000	RT-QP73-05	05-QP73-05	10-QP73-05
Takyon™ ROX Probe Core Kit dTTP		1250	UF-RPCT-C0201	UF-RPCT-C0205	UF-RPCT-C0210
qPCR Core kit dTTP		1000	RT-QP10-05NU	05-QP10-05NU	10-QP10-05NU
qPCR Core kit only dUTP		1000	RT-QP10-050U	05-QP10-050U	10-QP10-050U
qRT-PCR Probe Assays	**All above kits may be combined with our reverse transcriptase core kit RT-RTCK-03**				
One step qRT-PCR MasterMix	7.5 ml	600	RT-QPRT-032X	05-QPRT-032X	10-QPRT-032X

Table 2 - Kits with Low ROX passive reference - ABI Prism® 7500 & FAST 7500 • ViiA7™ • Mx3000P®

SYBR® Assays					
Takyon Low ROX SYBR MasterMix blue dTTP	7.5 ml	750	UF-LSMT-B0701	UF-LSMT-B0705	UF-LSMT-B0710
MESA GREEN qPCR MasterMix Plus for SYBR®	7.5 ml	600	RT-SY2X-03+WOULR	05-SY2X-03+WOULR	10-SY2X-03+WOULR
Assay Low ROX w/o UNG	15 ml	1200	RT-SY2X-06+WOULR	05-SY2X-06+WOULR	10-SY2X-06+WOULR
	50 ml	4000	RT-SY2X-20+WOULR	05-SY2X-20+WOULR	10-SY2X-20+WOULR
MESA BLUE qPCR MasterMix Plus for SYBR®	7.5 ml	600	RT-SY2X-03+WOULRB	05-SY2X-03+WOULRB	10-SY2X-03+WOULRB
qRT-PCR SYBR® Assays	**All above kits may be combined with our reverse transcriptase core kit RT-RTCK-03**				
For one-step assays, use the No ROX kit (see table 3) combined with ROX additive (RT-PARE-03)					
Probe Assays					
NEW Takyon™ Low ROX Probe MasterMix UNG	7.5 ml	750	UF-LPMU-C0701	UF-LPMU-C0705	UF-LPMU-C0710
NEW qPCR MasterMix Plus low ROX w/o UNG	7.5 ml	600	RT-QP2X-03+WOULR	05-QP2X-03+WOULR	10-QP2X-03+WOULR
NEW Takyon™ Low ROX Probe MasterMix dTTP BLUE	7.5 ml	750	UF-LPMT-B0701	UF-LPMT-B0705	UF-LPMT-B0710
qRT-PCR Probe Assays	**All above kits may be combined with our reverse transcriptase core kit RT-RTCK-03**				
One step qRT-PCR MasterMix Low ROX	7.5 ml	600	RT-QPRT-032XLR	05-QPRT-032XLR	10-QPRT-032XLR

[1] #RXNs is calculated for a final volume of 25 µl except for the Takyon™ Mixes and Core Kits for which the reaction is realised in a final volume of 20 µl.
RXNs is 5 and 10 times higher than the mentionned amount for 5 and 10-pack respectively.
[2] Combine with UNG RT-0610-03 for carryover prevention. Add UNG to the buffer bottle before first use.
Note : Easily find the best suited kit for your qPCR platform and application. Use our qPCR selector: www.eurogentec.com/qpcr-selector.html

* Sample for this kit is no longer available. Please evaluate the corresponding new Takyon™ kit. Combine with dUTP/UNG Sample (RT-UTPUNG-005) for carryover prevention.

• ABI Prism® 7700 • ABI Prism® 7900 & FAST 7900 • ABI Step One • ABI Step One Plus

Format	Full carryover (dUTP + UNG)	Maximal sensitivity (dTTP only)	FAST compatible	Corresponding sample
MasterMix	[2]			*
MasterMix	[2]			*
MasterMix	[2]			*
MasterMix		✔		*
MasterMix	[2]		✔	*
MasterMix	[3]	✔	✔	UF-RSMT-B0100
Core Kit	[2]			RT-SY2X-005 +WOU (MasterMix)
Core Kit		✔		RT-SY2X-005 +WOUN (MasterMix)
Core Kit	[3]	✔	✔	UF-RSMT-B0100 (MasterMix)
Core Kit	[2]			no sample
for optimal 2-step assays				
MasterMix				RT-SYRT-0052X
MasterMix	✔			RT-QP2X-005+
MasterMix	✔			RT-QP2X-005+
MasterMix	✔			RT-QP2X-005+
MasterMix	✔			RT-QP2X-005+
MasterMix	[2]			RT-QP2X-005+WOU
MasterMix		✔		RT-QP2X-005+WOUN
MasterMix	✔		✔	UF-RPMU-C0100
MasterMix	[3]	✔	✔	UF-RPMT-B0100
MasterMix	[3]	✔	✔	UF-RPMT-C0100
MasterMix	✔		✔	RT-QP2X-005+QGS
Core Kit	[2]			RT-QP2X-005+ (MasterMix)
Core Kit	[3]	✔	✔	UF-RPMT-B0100 (MasterMix)
Core Kit		✔		RT-QP2X-005+ (MMx with dUTP/UNG)
Core Kit	[2]			RT-QP2X-005+ (MMx with dUTP/UNG)
for optimal 2-step assays				
MasterMix		✔		RT-QPRT-0052X

• Mx3005P® • Mx4000®

MasterMix	[3]			UF-LSMT-B0100
MasterMix	[2]			*
MasterMix	[2]			*
MasterMix	[2]			*
MasterMix	[2]		✔	*
for optimal 2-step assays				
MasterMix	✔		✔	UF-LPMU-C0100
MasterMix	[2]			RT-QP2X-005+WOULR
MasterMix	[3]	✔	✔	UF-LPMT-B0100
for optimal 2-step assays				
MasterMix		✔		RT-QPRT-0052XNR + ROX (RT-PARE-03)

[3] Optional: when dUTP/UNG (RT-UTPUNG-020) additive is used. Automatically included in Core Kits.

Table 3 - Kits without ROX passive reference

DNA Engine Opticon® 1 & 2 • Chromo 4 • MiniOpticon • CFX96 & CFX384 • Rotor-Gene® 2000/3000/6000/Q • Quantica® • Mx4000® Swift Spectrum™

Product name		# RXNs per single kit 20 or 25µL[1]	Reference		
			Single kit	5-pack	10-pack
SYBR® Assays					
MESA GREEN qPCR MasterMix Plus for SYBR® Assay No ROX	7.5 ml	600	RT-SY2X-03+NRWOU	05-SY2X-03+NRWOU	10-SY2X-03+NRWOU
	15 ml	1200	RT-SY2X-06+NRWOU	05-SY2X-06+NRWOU	10-SY2X-06+NRWOU
	50 ml	4000	RT-SY2X-20+NRWOU	05-SY2X-20+NRWOU	10-SY2X-20+NRWOU
MESA BLUE qPCR MasterMix Plus for SYBR® Assay No ROX	7.5 ml	600	RT-SY2X-03+NRWOUB	05-SY2X-03+NRWOUB	10-SY2X-03+NRWOUB
NEW Takyon™ No ROX SYBR® MasterMix blue dTTP	7.5 ml	750	UF-NSMT-B0701	UF-NSMT-B0705	UF-NSMT-B0710
qPCR Core kit for SYBR® Green I No ROX		1000	RT-SN10-05NR	05-SN10-05NR	10-SN10-05NR
NEW Takyon No Rox SYBR Core Kit blue dTTP	7.5 ml	1250	UF-NSCT-B0201	UF-NSCT-B0205	UF-NSCT-B0210
qRT-PCR SYBR® Assays **All above kits may be combined with our reverse transcriptase core kit RT-RTCK-03**					
One step MESA GREEN qRT-PCR MasterMix for SYBR® Assay No ROX	7.5 ml	600	RT-SYRT-032XNR	05-SYRT-032XNR	10-SYRT-032XNR
Probe Assays					
NEW Takyon™ No ROX Probe MasterMix UNG	7.5 ml	750	UF-NPMU-C0701	UF-NPMU-C0705	UF-NPMU-C0710
NEW NEW Takyon™ No ROX Probe MasterMix dTTP BLUE	7.5 ml	750	UF-NPMT-B0701	UF-NPMT-B0705	UF-NPMT-B0710
Takyon™ No ROX Probe MasterMix dTTP	7.5 ml	750	UF-NPMT-C0701	UF-NPMT-C0705	UF-NPMT-C0710
qPCR MasterMix No ROX	7.5 ml	600	RT-QP2X-03NR	05-QP2X-03NR	10-QP2X-03NR
qPCR Core kit No ROX		1000	RT-QP73-05NR	05-QP73-05NR	10-QP73-05NR
NEW NEW Takyon™ No ROX Probe Core Kit dTTP		1250	UF-NPCT-C0701	UF-NPCT-C0705	UF-NPCT-C0710
Takyon™ No ROX Probe Core Kit dTTP BLUE		1250	UF-NPCT-B0701	UF-NPCT-B0705	UF-NPCT-B0710
qRT-PCR Probe Assays **All above kits may be combined with our reverse transcriptase core kit RT-RTCK-03**					
One step qRT-PCR MasterMix No ROX	7.5 ml	600	RT-QPRT-032XNR	05-QPRT-032XNR	10-QPRT-032XNR

Table 4 - Kits without ROX passive reference / with fluorescein

iCycler iQ® • My iQ • iQ™5

SYBR® Assays with fluorescein additive					
Takyon SYBR MasterMix blue dTTP with fluorescein	7.5 ml	750	UF-FSMT-B0701	UF-FSMT-B0705	UF-FSMT-B0710
MESA GREEN qPCR MasterMix Plus for SYBR® Assay w/fluorescein	7.5 ml	600	RT-SY2X-03+WOUFL	05-SY2X-03+WOUFL	10-SY2X-03+WOUFL
	15 ml	1200	RT-SY2X-06+WOUFL	05-SY2X-06+WOUFL	10-SY2X-06+WOUFL
	50 ml	4000	RT-SY2X-20+WOUFL	05-SY2X-20+WOUFL	10-SY2X-20+WOUFL
MESA BLUE qPCR MasterMix Plus for SYBR® Assay w/ fluorescein	7.5 ml	600	RT-SY2X-03+WOUFLB	05-SY2X-03+WOUFLB	10-SY2X-03+WOUFLB
qPCR MasterMix Plus for SYBR® green I w/fluorescein	7.5 ml	600	RT-SN2X-03+NRFL	05-SN2X-03+NRFL	10-SN2X-03+NRFL
qRT-PCR SYBR® Assays **All above kits may be combined with our reverse transcriptase core kit RT-RTCK-03**					
One step MESA GREEN qRT-PCR MasterMix for SYBR® Assay No ROX[4]	7.5 ml	600	RT-SYRT-032XNR	05-SYRT-032XNR	10-SYRT-032XNR
Probe Assays					
NEW NEW NEW Takyon™ No ROX Probe MasterMix UNG	7.5 ml	750	UF-NPMU-C0701	UF-NPMU-C0705	UF-NPMU-C0710
Takyon™ No ROX Probe MasterMix dTTP BLUE	7.5 ml	750	UF-NPMT-B0701	UF-NPMT-B0705	UF-NPMT-B0710
Takyon™ No ROX Probe MasterMix dTTP	7.5 ml	750	UF-NPMT-C0701	UF-NPMT-C0705	UF-NPMT-C0710
qPCR MasterMix No ROX	7.5 ml	600	RT-QP2X-03NR	05-QP2X-03NR	10-QP2X-03NR
NEW NEW qPCR Core kit No ROX[4]		1000	RT-QP73-05NR	05-QP73-05NR	10-QP73-05NR
Takyon™ No ROX Probe Core Kit dTTP		1250	UF-NPCT-C0201	UF-NPCT-C0205	UF-NPCT-C0210
Takyon™ No ROX Probe Core Kit dTTP BLUE		1250	UF-NPCT-B0201	UF-NPCT-B0205	UF-NPCT-B0210
qRT-PCR Probe Assays **All above kits may be combined with our reverse transcriptase core kit RT-RTCK-03**					
One step qRT-PCR MasterMix No ROX	7.5 ml	600	RT-QPRT-032XNR	05-QPRT-032XNR	10-QPRT-032XNR

[1] #RXNs is calculated for a final volume of 25µl except for the Takyon™ Mixes and core kits for which the reaction is realised in a final volume of 20µl. RXNs is 5 and 10 times higher than the mentionned amount for 5 and 10-pack respectively.
[2] Combine with UNG RT-0610-03 for carryover prevention. Add UNG to the buffer bottle before first use.
Note : Easily find the best suited kit for your qPCR platform and application. Use our qPCR selector: www.eurogentec.com/qpcr-selector.html

* Sample for this kit is no longer available. Please evaluate the corresponding new Takyon™ kit. Combine with dUTP/UNG Sample (RT-UTPUNG-005) for carryover prevention.

• Mx3000P® • Mx3005P® • SmartCycler® 1 & 2 • LC96/LC480 Mastercycler® ep realplex I & II • LightCycler™ Nano

Format	Full carryover (dUTP + UNG)	Maximal sensitivity (dTTP only)	FAST compatible	Corresponding sample
MasterMix	[2]			*
MasterMix	[2]			*
MasterMix	[2]			*
MasterMix	[2]		✔	*
MasterMix	[3]	✔	✔	UF-NSMT-B0100
Core Kit	[2]			no sample
Core Kit	[3]	✔	✔	
for optimal 2-step assays				
MasterMix				RT-SYRT-0052XNR
MasterMix	✔		✔	UF-NPMU-C0100
MasterMix	[3]	✔	✔	UF-NPMT-B0100
MasterMix	[3]	✔	✔	UF-NPMT-C0100
MasterMix	✔			RT-QP2X-005+NR
Core Kit	[2]			RT-QP2X-005+NR (MasterMix)
Core Kit	[3]	✔	✔	UF-NPMT-C0100 (MasterMix)
Core Kit	[3]	✔	✔	UF-NPMT-B0100 (MasterMix)
for optimal 2-step assays				
MasterMix		✔		RT-QPRT-0052XNR

MasterMix	[3]	✔	✔	
MasterMix	[2]			*
MasterMix	[2]			*
MasterMix	[2]			*
MasterMix	[2]		✔	*
MasterMix	✔			no sample
for optimal 2-step assays				
MasterMix		✔	✔	RT-SYRT-0052XNR
MasterMix	✔		✔	UF-NPMU-C0100
MasterMix	[3]	✔	✔	UF-NPMT-B0700
MasterMix	[3]	✔	✔	UF-NPMT-C0100
MasterMix	✔			RT-QP2X-005+NR
Core Kit	[2]			RT-QP2X-005+NR (MasterMix)
Core Kit	[3]	✔	✔	UF-NPMT-C0100 (MasterMix)
Core Kit	[3]	✔	✔	UF-NPMT-B0100 (MasterMix)
for optimal 2-step assays				
MasterMix		✔		RT-QPRT-0052XNR

[3] Optional: when dUTP/UNG (RT-UTPUNG-020) additive is used. Automatically included in Core Kits.
[4] Combine with fluorescein additive (RT-FLUO-ADD)

All Techniques

- SYBR® assays
- Probe assays
- DNA/cDNA/gDNA detection
- RNA detection
- One or 2 Step qRT-PCR

Cost efficient

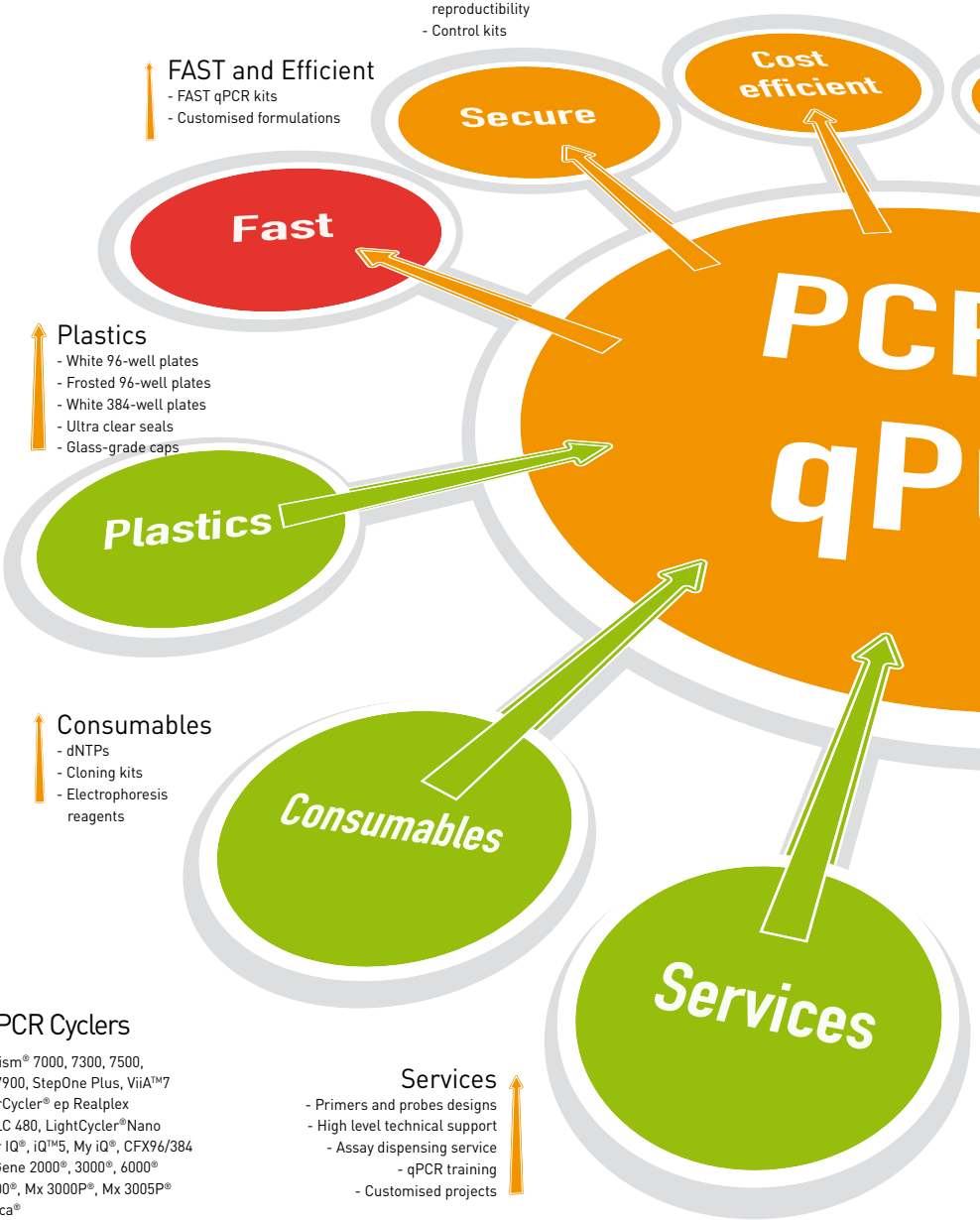
- 5 & 10 pack bundles
- Large volume kits (50 ml)
- Core kit format
- Assay dispensing service
- Batch consigment for simplified SOPs

Secure

- Kits with dUTP/UNG blend for contamination prevention
- Batch consignments for maximal reproductibility
- Control kits

FAST and Efficient

- FAST qPCR kits
- Customised formulations



Plastics

- White 96-well plates
- Frosted 96-well plates
- White 384-well plates
- Ultra clear seals
- Glass-grade caps

Consumables

- dNTPs
- Cloning kits
- Electrophoresis reagents

All qPCR Cyclers

- ABI Prism® 7000, 7300, 7500, 7700, 7900, StepOne Plus, ViiA™7
- MasterCycler® ep Realplex
- LC96/LC 480, LightCycler®Nano
- ICycler IQ®, iQ™5, My iQ®, CFX96/384
- RotorGene 2000®, 3000®, 6000®
- Mx 4000®, Mx 3000P®, Mx 3005P®
- Quantica®
- SmartCycler® 1&2
- Opticon® 1&2, Chromo 4, MiniOpticon
- Swift Spectrum™ 96

Services

- Primers and probes designs
- High level technical support
- Assay dispensing service
- qPCR training
- Customised projects

All Applications

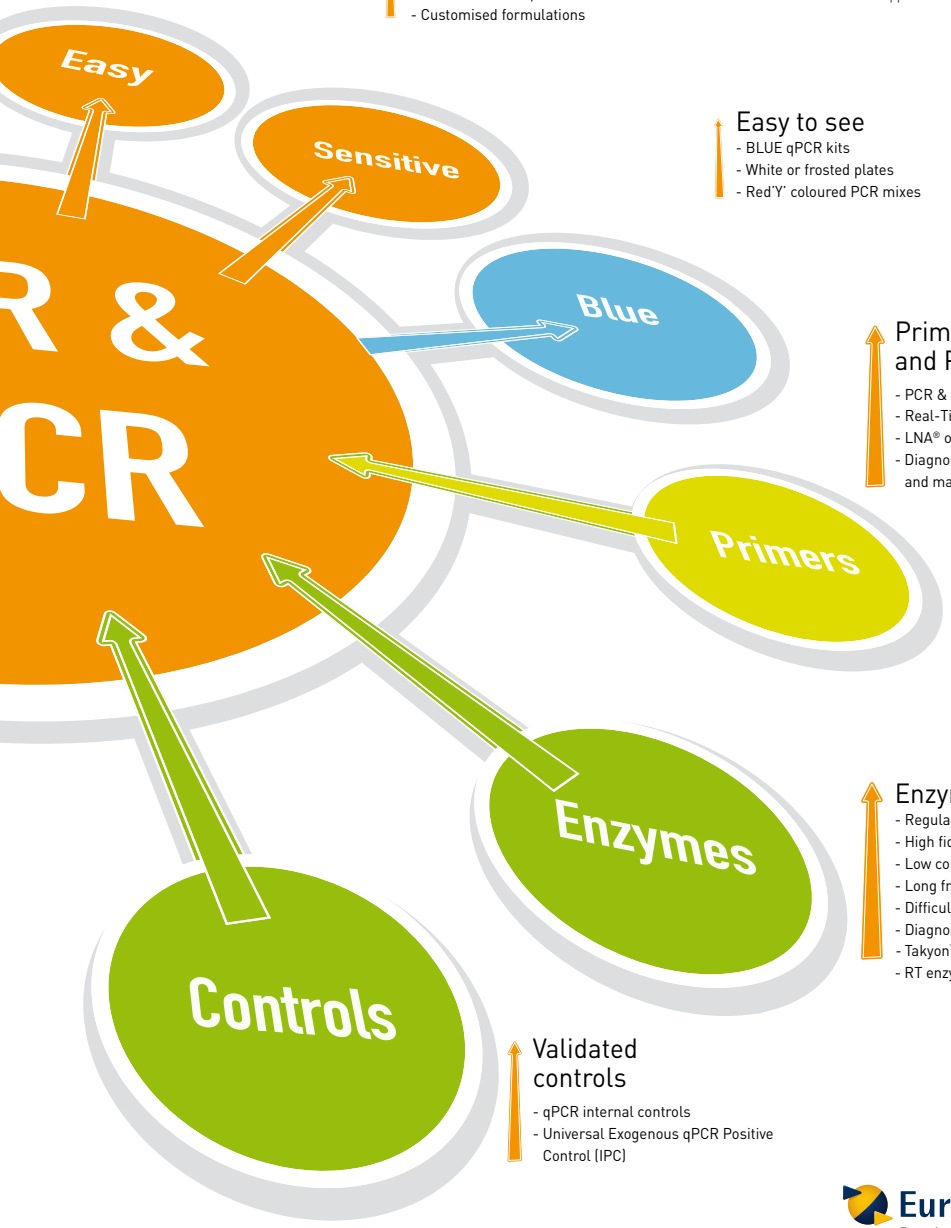
- Gene expression
- Genotyping/SNPs
- miRNA qRT-PCR
- Viral detection
- Multiplex detection
- Gene scanning
- ChIP/methylation qPCR
- Sequencing and all PCR applications

Ready and Easy to use

- Optimised qPCR MasterMixes
- PCR "Ready" mixes
- Assay dispensing service
- Optimised packaging

Sensitive

- dTTP qPCR kits
- QuickGoldStar® qPCR kits
- Customised formulations



Easy to see

- BLUE qPCR kits
- White or frosted plates
- Red'Y' coloured PCR mixes

Primers and Probes

- PCR & qPCR primers
- Real-Time qPCR probes
- LNA® oligos
- Diagnostic oligos and many more...

Enzymes

- Regular & HotStart *Taq*
- High fidelity *Taq*
- Low copy *Taq*
- Long fragment *Taq*
- Difficult template *Taq*
- Diagnostic and GMP *Taq*
- Takyon™ Enzyme
- RT enzymes

Validated controls

- qPCR internal controls
- Universal Exogenous qPCR Positive Control (IPC)

Eurogentec
Experience true partnership

Take advantage of a true flexibility with Eurogentec

Custom MasterMix!

Because setting up a qPCR assay can be a real challenge requiring an optimised mix composition, Eurogentec offers you the possibility to produce your own qPCR Mix.

The minimal ordered volume is 200ml. We guarantee a 1 and 2 years of stability for SYBR® and Probe MasterMixes, respectively.

Combine the following advantages in a single product:

- Maximized sensitivity and specificity of your custom validated MasterMix.
- Absolute reproducibility with the benefits of a unique batch.
- Minimal contamination risks and pipetting errors with the ready-to-use MasterMix.
- Consignement and scheduled deliveries.

License statements

UDG

Use of UDG employs U.S. Patents 5,035,996, 5,945,313, 5,683,896 and their foreign counterparts licensed to Eurogentec S.A. from Invitrogen Corporation. Cyclic-substituted unsymmetrical cyanine dyes are covered by U.S. Patents 5,436,134 and 5,658,751 and licensed to Eurogentec S.A. by Molecular Probes, Inc. in the direct research field.

SYBR® Green 1 Nucleic acid gel stain

Cyclic-substituted unsymmetrical cyanine dyes are covered by U.S. Patents 5,436,134 and 5,658,751,and licensed to Eurogentec S.A. by Molecular Probes, Inc. in the direct research field.

Chemically-Modified HotStart Polymerase Reagents and Kits

Purchase of these products includes an immunity from suit under patents specified in the product insert to use only in the amount purchased for the purchaser's own internal research. No other patent rights are conveyed expressly, by implication or by estoppel. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Center Drive, Foster City ,California 94 404, USA.

Trademarks

ABI Prism® 5700, 7000, 7500, 7700 and 7900 are registered trademarks of Applied Biostystems.
iCycler iQ® is a registered trademark of Bio-Rad Laboratories, Inc.
iCycler® is a registered trademark of Bio-Rad Laboratories, Inc.
iQ™5 is a trademark of Bio-Rad Laboratories, Inc.
LightCycler® is a registered trademark of Roche Molecular Systems.
Mastercycler® is a registered trademark of Eppendorf, Inc.
Mx3000P®, Mx3005P®, Mx4000® are registered trademark of Stratagene.
Opticon® is a registered trademark of MJ Research, Inc.
Quantica® is a registered trademark of Techne Ltd and Techne, Inc.
Rotor-Gene® is a registered trademark of Corbett Research Pty Ltd.
SmartCycler® is a registered trademark of Cepheid, Inc.
SYBR® is a registered trademark of Molecular Probes, Inc. (a wholly owned subsidiary of Invitrogen Corp.)
Taqman® is a registered trademark of Applied Biosystems, Inc.
Takyon™ is a registered trademark of Eurogentec.
ViiA™7 is a registered trademark of Life Technologies.

©FOTOLIA

Customer Support

Thank you for this first step toward the validation of your new qPCR MasterMix.
We stay at your side during the sample validation phase, so don't hesitate to contact us.
Within 5 weeks from sample delivery, one of our account managers will contact you to enquire about your satisfaction with this sample and to activate your **Discovery Offer** on your first kit order corresponding to this sample.

	Europe	US
@	info@eurogentec.com	tech@anaspec.com
☎	Toll free number: 00800 666 00 123	Toll free number: +1-800-452-5530

Kits and Reagents ordering

Place your order online via the Eurogentec Ordering System (EOS) interface.
Simply register and log in on EOS platform.

EOS	www.eurogentec.com	www.anaspec.com
@	order@eurogentec.com	service@anaspec.com
🖷	+32 (0)4 264 07 88	+1-510-791-9572

Eurogentec
Experience true partnership