

## LightMix® Kit *CYP2C19\*2* and *CYP2C19\*3*

Cat.-No. 40-0304-32

Detection Kit with reagents for the detection of alleles *CYP2C19\*2* and *CYP2C19\*3* in human DNA using the Roche Diagnostics LightCycler® 1.x / 2.0 / 480 and cobas z 480 Analyzer.

Lyophilized mix of primers and probes for a total of 96 reactions with a final volume of 20 µl each.  
**Store protected from light at room temperature (18-25°C), do NOT freeze!**

Instructions for use with the LightCycler® 1.x / 2.0 Instruments see pages 4-5

Instructions for use with the LightCycler® 480 and cobas z 480 Instruments see pages 6-7

### 1. Introduction

Cytochrome P450 (CYP) enzymes are monooxygenases primarily localized in the liver. They are in particular important for oxidation reactions in drug metabolism. Cytochrome 2C19 metabolizes commonly prescribed drugs like Diazepam, Amitriptylin and Propranolol. *CYP2C19* belongs to the CYP2C subfamily, including also the isoenzymes CYP2C8, CYP2C9 and CYP2C18.

The gene is polymorphic. Within more than 20 described variants ([www.cypalleles.ki.se/cyp2c19.htm](http://www.cypalleles.ki.se/cyp2c19.htm)) alleles \*2 and \*3 are more important since they are quite common and cause a loss of enzyme activity. For *CYP2C19\*2* (rs4244285, up to 10% frequency) substitution of base c.681G>A causes a splicing defect thus changing the protein (p.Pro227=), while for *CYP2C19\*3* (rs4986893, ~5%) substitution of base c.636G>A creates an earlier stop codon causing a truncated protein (p.Trp212Ter).

This kit provides a fast and accurate method to identify the *CYP2C19* genotype from a nucleic acid extract. The kit has been tested with 'LightCycler® FastStart DNA Master HybProbe' only.

### 2. Description

Two 133 and 164 bp long fragments of the human *CYP2C19* gene are amplified with specific primers. Allele \*2 is detected with a SimpleProbe® oligomer (detected in channel 530) and allele \*3 is detected with LightCycler® Red 640 labeled probes (detected in channel 640).

The genotypes are identified by running a melting curve and reading the specific melting points (T<sub>m</sub>).

With LightCycler® 2.0 Instruments the wildtype *CYP2C19\*1* exhibits a T<sub>m</sub> of 54.4°C in channel 530 and 53.4°C in channel 640. The allele \*2 exhibits a T<sub>m</sub> of 48.6°C in channel 530, and the allele 3 yields a T<sub>m</sub> of 60.8°C in channel 640. For plate based Instruments the wildtype *CYP2C19\*1* exhibits a T<sub>m</sub> of 53.8°C in filter 483-533 and a T<sub>m</sub> of 52.4°C in filter 483-640 while the allele \*2 exhibits a T<sub>m</sub> of 48.1°C in filter 483-533, and the allele \*3 a T<sub>m</sub> of 59.2°C in filter 483-640.

The use of a color compensation file generated with the LightMix® – Assay Color Compensation 530/640/690 is a prerequisite for identifying the genotypes in the corresponding channel.

The supplied control DNAs allow for the accurate comparison with unknown samples.

### 3. Set Contents

- 3 Vials with **red** cap containing premixed lyophilized primers and probes for 32 reactions
- 1 Vial with **colorless** cap containing *CYP2C19\*2* reaction wild type control DNA (10<sup>5</sup> per reaction)
- 1 Vial with **colorless** cap containing *CYP2C19\*2* reaction mutant control DNA (10<sup>5</sup> per reaction)
- 1 Vial with **colorless** cap containing *CYP2C19\*2* reaction hetero control DNA (10<sup>5</sup> per reaction)
- 1 Vial with **colorless** cap containing *CYP2C19\*3* reaction wild type control DNA (10<sup>5</sup> per reaction)
- 1 Vial with **colorless** cap containing *CYP2C19\*3* reaction mutant control DNA (10<sup>5</sup> per reaction)
- 1 Vial with **colorless** cap containing *CYP2C19\*3* reaction hetero control DNA (10<sup>5</sup> per reaction)

## 4. Additional Reagents and items required

Roche Diagnostics

Color Compensation HybProbe order n°40-0318-00	Cat.-No. 05 997 704 001
LightCycler® FastStart DNA Master HybProbe	Cat.-No. 03 003 248 001
LightCycler® Capillaries (20 µl) (LightCycler® 1.x / 2.0 Instruments)	Cat.-No. 04 929 292 001
LightCycler® 480 Multiwell Plate 384, white (LightCycler® 480 Instrument)	Cat.-No. 04 729 749 001
or LightCycler® 480 Multiwell Plate 96, white (LightCycler® 480 Instrument)	Cat.-No. 04 729 692 001

For use in LightCycler® 1.x Instruments with software version 3.5.3 read channel F2 instead of channel 640, channel F3 instead of channel 705 and channel F1 instead of channel 530 for detection. We recommend upgrading LightCycler® 1.x Instruments to software version 4.1.

For use in LightCycler® 480 Instruments use filter combination 483-533 (LightCycler® 480 II Instrument filter combination 465-510) instead of channel 530 and filter combination 483-640 (LightCycler® 480 II Instrument filter combination 498-640) instead of channel 640 for detection.

### 4.1. Optional Additional Reagents

High Pure PCR Template Preparation Kit	Cat.-No. 11 796 828 001
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## 5. Product Characteristics

PCR results (activation, 50 cycles and melting curve) are obtained within 50 minutes with the capillary based LightCycler® 1.x / 2.0 Instruments and within 80 minutes with '480' plate based Instruments.

### Sensitivity

These reagents detect the variation with 1 ng human DNA using the Roche 'LightCycler® FastStart DNA Master HybProbe' (in an exemplary system, using cloned targets as reference).

### Measuring range

Allowed range of target DNA is 10 to 100 ng using 'LightCycler® FastStart DNA Master HybProbe'.

### Storage and Stability

- Lyophilized reagents are stable for at least 6 months after shipment when stored protected from light at room temperature (18-25°C). See expiry date on the product label.
- **Do not freeze** lyophilized reagents.
- Dissolved reagents are stable for at least 10 days when stored protected from light and refrigerated (4°C).

## 6. Experimental Protocol

The following procedure was developed for use with the LightCycler® 1.x / 2.0 / 480 Instruments. Start programming before preparing the solutions. See the Instrument operator's manual for details.

**Sample material:** Use aqueous nucleic acid preparations (e.g. 'High Pure PCR Template Preparation Kit' for genomic DNA samples).

**Negative control:** Always run at least one no-template control (NTC) - replace the template DNA with water.

**Positive control:** Run a positive control - replace the template DNA with the provided control DNA.

### 6.1. Preparation of parameter-specific reagents (32 reactions):

One reagent vial with a **red** cap contains primers and probes to run 32 reactions.

**Add 66 µl** PCR-grade water to each reagent vial, mix the solution (vortex) and spin down.

► **Use 2 µl** reagent for a 20 µl PCR reaction.

| This solution is stable at least five days when stored refrigerated at 4°C. Avoid prolonged exposure to light.

### 6.2. Preparation of the control DNA

Add 80 µl PCR-grade water to each vial ( $1.6 \times 10^6$  target molecules) with a **colorless** cap. Mix the target DNA by pipetting the solution up and down 10 times.

**Note:** Control DNA can be dissolved up to 160 µl to achieve 32 control reactions.

► **Use 5 µl** control DNA for a 20 µl PCR reaction.

| This solution is stable at least five days when stored refrigerated at 4°C, for long term storage freeze at -20°C. Avoid repeated freezing thawing cycles. For the heterozygote control DNA provided with the kit please note that the relative amounts of wild type DNA and mutant DNA may change during time.

| Please note that opening these vials may cause contaminations of the work-space (aerosol).

### 6.3. Preparation of the reaction mix

In a cooled reaction tube, prepare the reaction mix by multiplying each volume for a single reaction by the number of reactions to be cycled plus one additional reaction.

For use with the Roche FastStart Master	
Single reaction	Component
9.4 µl	water, PCR-grade (colorless cap, provided with the Roche Master kit)
1.6 µl	Mg <sup>2+</sup> solution 25 mM (blue cap, provided with the Roche FastStart kit)
2.0 µl	<b>reagent</b> mix (parameter specific reagents containing primers and probes, see 6.1.)
2.0 µl	Roche Master (red cap, for preparation see Roche manual)

**15.0 µl**

Volume of reaction mix

Table 1

Mix gently, spin down and **transfer 15 µl** of the reaction mix to a capillary or well.

**Add 5 µl** of sample or standard to each capillary or well for a final reaction volume of 20 µl. Close the capillaries / attach a foil to the multiwell plate and seal, and spin down.

**Start run.**

## 7. LightCycler® 1.x / 2.0 Instruments

### 7.1. Programming

The protocol consists of four program steps

- 1: Denaturation: sample denaturation and enzyme activation
- 2: Cycling: PCR-amplification of the target DNA
- 3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
- 4: Cooling: cooling the instrument

Program Step:	Denaturation	Cycling			Melting			Cooling
<b>Parameter</b>								
Analysis Mode	None	Quantification mode			Melting Curves mode			None
Cycles	1	45			1			1
Target [°C]	95	95	60	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:10	00:00:15	00:00:20	00:00:20	00:00:00	00:00:30
Ramp Rate [°C/s]	20	20	20	20	20	20	0.2	20
Acquisition Mode	None	None	Single	None	None	None	Continuous	None

Table 2

### 7.2. Data Analysis

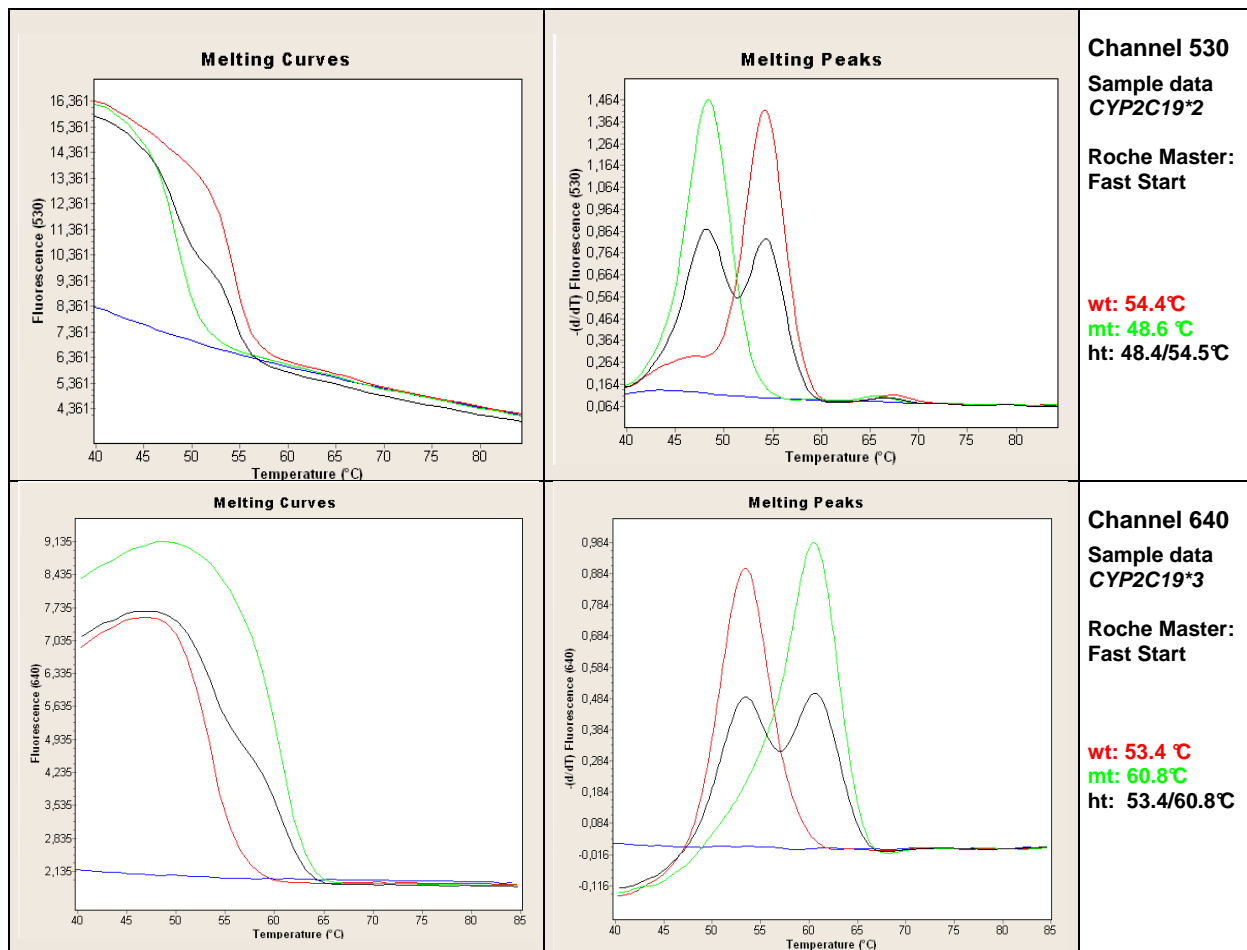
For use in LightCycler® 1.x Instruments with software version 3.5.3 read channel F2 instead of channel 640 and channel F1 instead of channel 530 for detection. We recommend upgrading LightCycler® 1.x instruments to software version 4.1.

Switch the color compensation mode on. If this mode is not enabled run the color compensation program. Follow the instructions in the manual of the Color Compensation Kit HybProbe 530/640/690'. Perform data analysis, as described in the LightCycler® Instrument operator's manual.

View *CYP2C19\*3* data in channel 640 and *CYP2C19\*2* data in channel 530, "Tm Calling" Analysis mode (LightCycler® 2.0 Instrument) or Melting Curves mode (LightCycler® 1.x Instrument).

The negative control (NTC) must show no signal.

### 7.3. Sample Data – Typical Results



**Fig.1.** LightCycler® 2.0 sample data for the *CYP2C19\*2* and *CYP2C19\*3* detection system.

**Upper panels:** Data from channel 530. Left panel melting curves for *CYP2C19\*2*. Right panel melting peaks for *CYP2C19\*2*. Wildtype (wt) corresponds with *CYP2C19\*1/\*1*, heterozygous (ht) with *\*1/\*2* and mutant (mt) corresponds with *\*2/\*2*.  
**Lower panels:** Data from channel 640. Left panel melting curves for *CYP2C19\*3*. Right panel melting peaks for *CYP2C19\*3*. Wildtype (wt) corresponds with *CYP2C19\*1/\*1*, heterozygous (ht) with *\*1/\*3* and mutant (mt) corresponds with *\*3/\*3*.

### 7.4. Interpretation of Data

*2 Channel 530 Melting point(s)		*3 Channel 640 Melting point(s)		CYP 2C19 alleles	Metabolizers Phenotype
-	54.4°C	53.4°C	-	*1/*1 (wild type)	Extensive
48.6°C	54.4°C	53.4°C	-	*1/*2	Intermediate
-	54.4°C	53.4°C	60.8°C	*1/*3	Intermediate
48.6°C	-	53.4°C	-	*2/*2	slow (inactive)
-	54.4°C	-	60.8°C	*3/*3	slow (inactive)
48.6°C	54.4°C	53.4°C	60.8°C	*2/*3	slow (inactive)
48.6°C	-	-	60.8°C	never observed	slow (inactive)
-	-	-	-	PCR failure	<b>Repeat test</b>
ΔTm 6°C		ΔTm 8°C			

**Table 3.** Typical analysis results (LightCycler® 2.0 Instrument, Roche Master: Fast Start)

**Notes:** The values of the respective melting temperatures ( $T_m$ ) may vary  $\pm 2.5^\circ\text{C}$  between different experiments. The  $\Delta T$  between the melting peaks for heterozygote genotypes may vary  $\pm 1.5^\circ\text{C}$ . Samples with deviating melting curves should be subject to further investigations; sequence analysis can be provided by TIB MOLBIOL Berlin (contact [service@tib-molbiol.de](mailto:service@tib-molbiol.de)).

## 8. LightCycler® 480 Instruments and cobas z 480 Analyzer

### 8.1. Programming

The protocol consists of four program steps

- 1: Denaturation: sample denaturation and enzyme activation
- 2: Cycling: PCR-amplification of the target DNA
- 3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
- 4: Cooling: cooling the instrument

Detection Format:

LightCycler® 480 Instrument: 483-533, 483-640

LightCycler® 480 II Instrument: 465-510, 498-640

cobas z 480 Analyzer: 465-510, 498-645

Program Step:	Denaturation	Cycling			Melting			Cooling
Parameter								
Analysis Mode	None	Quantification mode			Melting Curves mode			None
Cycles	1	45			1			1
Target [°C]	95	95	60	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:10	00:00:15	00:00:30	00:02:00	00:00:00	00:00:30
Ramp Rate [°C/s] 96	4.4	4.4	2.2	4.4	4.4	1.5	-	1.5
Ramp Rate [°C/s] 384	4.6	4.6	2.4	4.6	4.6	2.0	-	2.0
Acquisition Mode	None	None	Single	None	None	None	Continuous	None
Acquisitions [per °C]	-	-	-	-	-	-	1	-

Table 4

### 8.2. Data Analysis

**Note:** cobas z 480 analyzer signal levels are about 50% compared to LightCycler® 480 II results.

Note: For use on LightCycler® 480 II Instruments select Filter Combination 465-510 instead of Filter Combination 483-533 and Filter Combination 498-640 instead of Filter Combination 483-640 for detection.

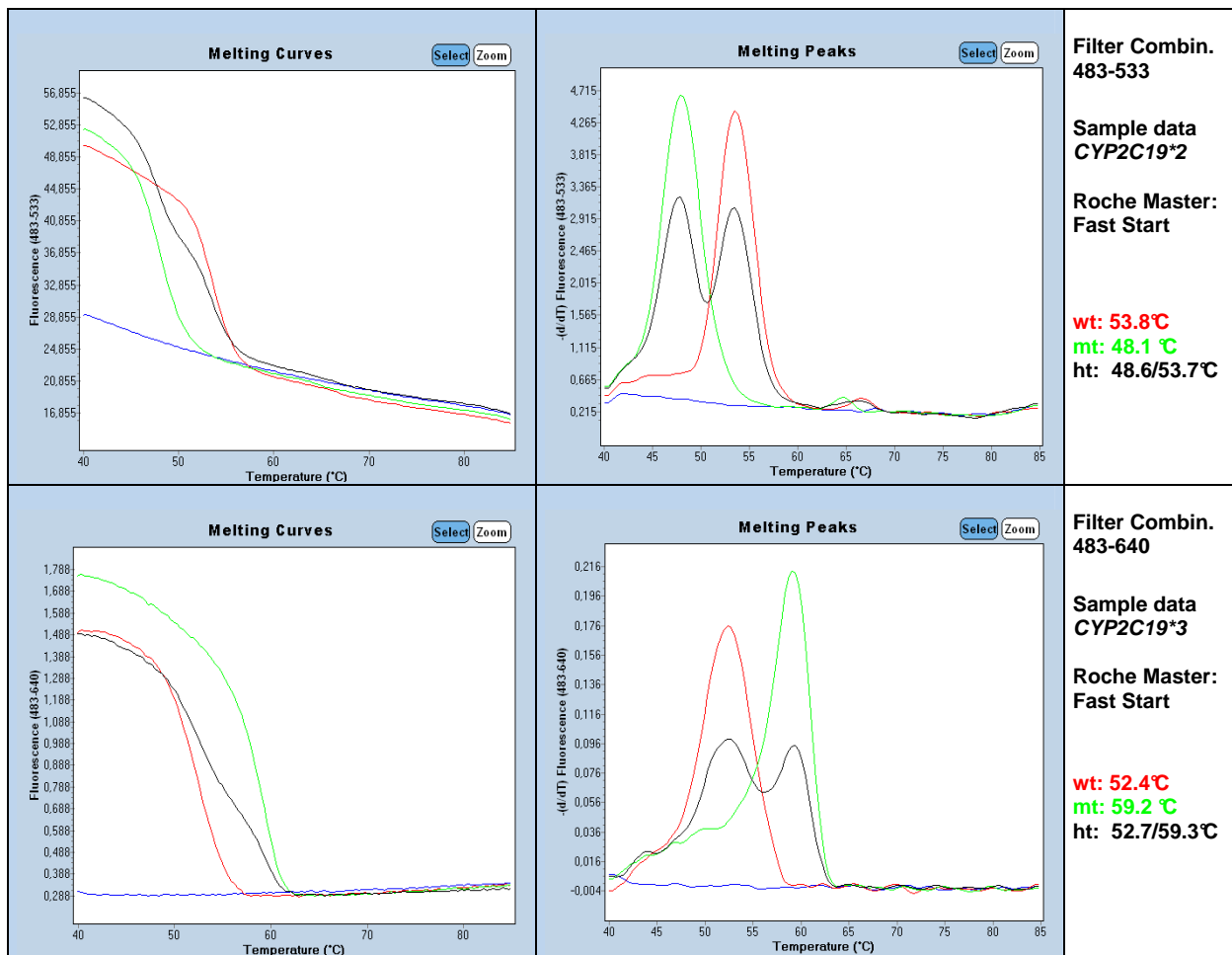
Switch the color compensation mode on. If this mode is not enabled run the color compensation program. Follow the instructions in the manual of the Color Compensation Kit HybProbe 530/640/690'.

Perform data analysis, as described in the LightCycler® Instrument operator's manual.

View CYP2C19\*2 data with Filter Combination 483-533 and CYP2C19\*3 data with Filter Combination 483-640, "Tm Calling" Analysis mode.

The negative control (NTC) must show no signal.

### 8.3. Sample Data – Typical Results



**Fig 2.** LightCycler® 480 sample data for the human *CYP2C19\*2* and *CYP2C19\*3* detection system.

**Upper panels:** Left panel Filter Combination 483-533 melting curves for \*2. Right panel filter 483-533 melting peaks for \*2. Wildtype (wt) corresponds with *CYP2C19\*1/\*1*, heterozygous (ht) with \*1/\*2 and mutant (mt) corresponds with \*2/\*2.  
**Lower panels:** Left panel Filter Combination 483-640 melting curves for \*3. Right panel filter 483-640 melting peaks for \*3. Wildtype (wt) corresponds with *CYP2C19\*1/\*1*, heterozygous (ht) with \*1/\*3 and mutant (mt) corresponds with \*3/\*3.

### 8.4. Interpretation of Data

*2 Filter 483-533 Melting point(s)		*3 Filter 483-640 Melting point(s)		CYP 2C19 alleles	Metabolizers Phenotype
-	53.8°C	52.4°C	-	*1/*1 (wild type)	Extensive
48.1°C	53.8°C	52.4°C	-	*1/*2	Intermediate
-	53.8°C	52.4°C	59.2°C	*1/*3	Intermediate
48.1°C	-	52.4°C	-	*2/*2	slow (inactive)
-	53.8°C	-	59.2°C	*3/*3	slow (inactive)
48.1°C	53.8°C	52.4°C	59.2°C	*2/*3	slow (inactive)
48.1°C	-	-	59.2°C	never observed	slow (inactive)
-	-	-	-	PCR failure	<b>Repeat test</b>
ΔTm 6°C		ΔTm 8°C			

**Table 5.** Typical analysis results (LightCycler® 480 Instrument, Roche Master: Fast Start)

**Notes:** The values of the respective melting temperatures ( $T_m$ ) may vary  $\pm 2.5^\circ\text{C}$  between different experiments. The  $\Delta T$  between the melting peaks for heterozygote genotypes may vary  $\pm 1.5^\circ\text{C}$ . Samples with deviating melting curves should be subject to further investigations; sequence analysis can be provided by TIB MOLBIOL Berlin (contact [service@tib-molbiol.de](mailto:service@tib-molbiol.de)).

## 9. Material Safety Data

According to OSHA 29CFR1910.1200, Commonwealth of Australia [NOHSC:1005, 1008 (1999)] and the European Union Directives 67/548/EC and 1999/45/EC any products which not contain more than 1% of a component classified as hazardous or classified as carcinogenic do not require a Material Safety Data Sheet (MSDS).

Product is not hazardous, not toxic, not IATA-restricted. Product is not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes.

## 10. Version History

Notes in red mark events require to change procedures

V080304  
V110218  
V110321  
V120527  
V140414

EAN number included  
Double amount of control DNA and 32rxn/vial  
Instructions for the use LC 480 instruments  
Corrections 8.4 Interpretation Table LC480  
MSDS included, Editorial changes

Roche SAP order n° 05945739001

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These reagents were developed and manufactured by TIB MOLBIOL GmbH, Berlin, Germany.  
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