LightMix® Kit beta-Globin Extraction Control

Cat.-No. 40-0085-32

Kit for the detection / estimation of the quantity of human genomic DNA targeting the ß-Globin gene using the Roche Diagnostics LightCycler® 1.x / 2.0 / 480 Instruments or 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℃), do NOT freeze!**

1. Introduction

Pathogen-negative NAT test results require to ensure absence of PCR inhibition and evidence that the specimen had been from human origin and extracted properly. One option for verification of diagnostic results is the amplification of a(ny) human gene. The ß-Globin gene is a preferred target for control PCR assays. This assay is intended to be run in a separate reaction and not in a multiplex reaction.

Quantification of nucleic acids is usually based on a spectrophotometric measurement. For degraded materials such as DNA extracted from FFPE samples the amount of amplificable DNA might differ from the result from the spectrophotometric measurement and can be checked using this kit.

This assay can be calibrated with human DNA of known concentration to quantify unknown samples.

Reference:

Continuous fluorescence monitoring of rapid cycle DNA amplification. Wittwer CT, Herrmann MG, Moss AA, Rasmussen RP. Biotechniques. 1997 134-8

2. Description

A 143 bp fragment of the human beta globin gene is amplified wth specfic primers and detected with hybridization probes labeled with LightCycler® Red 640, detected in channel 640.

For use with LightCycler® 1.x Instruments use channel F2, for 480 systems use filters combinations :

LightCycler® 480 Instrument	483-640
LightCycler® 480 II Instrument:	498-640
cobas z 480 Analyzer:	498-645

This kit is tested for use with the Roche 'LightCycler® FastStart DNA Master HybProbe' only.

3. Set Contents

3 Vials with red caps containing premixed lyophilized primers and probes for 32 PCR reactions

4. Additional Reagents and Items Required

	Roche Diagnostics
LightCycler® FastStart DNA Master HybProbe	Cat.-No. 03 003 248 001
LightCycler® Capillaries (20 µl) (LightCycler® 1.x/2 Instruments)	Cat.-No. 04 929 292 001
LightCycler® 480 Multiwell Plate 96, white (480 Instruments)	Cat.-No. 04 729 692 001

5. Product Characteristics

PCR results are obtained within 1 hour.

Sensitivity
These reagents detect 0.01 ng of β-*Globin* genomic DNA.

Measuring range
The linear measuring range of the assay is 0.0 1 ng to 100 ng of β-*Globin* DNA.

Storage and Stability
- Lyophilized reagents are stable for at least 6 months after shipment if stored protected from light at room temperature (18-25℃).
- **Do not freeze** lyophilized reagents.
- Dissolved reagents are stable for at least 10 days if stored protected from light and refrigerated (4℃).

6. 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
Parameter								
Analysis Mode	None	Quantification mode			Melting Curves mode			None
Cycles	1	45			1			1
Target [℃]	95	95	55	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:10	00:00:20	00:00:30	00:00:20	00:00:20	00:00:00	00:00:30
Ramp Rate [℃/s]	20	20	20	20	20	20	0.2	20
Acquisition Mode	None	None	Single	None	None	None	Continuous	None
Acquisitions [per ℃]	-	-	-	-	-	-	1	-

Program Step:	Denaturation	Cycling			Melting			Cooling
Parameter								
Analysis Mode	None	Quantification mode			Melting Curves mode			None
Cycles	1	45			1			1
Target [℃]	95	95	55	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:10	00:00:20	00:00:30	00:00:30	00:02:00	00:00:00	00:00:30
Ramp Rate [℃/s] **96**	4.4	4.4	2.2	4.4	4.4	1.5	-	1.5
Ramp Rate [℃/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 ℃]	-	-	-	-	-	-	1	-

(Melting not relevant for detection) Table 1

This assay might be used with any other cycler programs using an annealing temperature 50℃-60℃ (has to be tested experimentally before using in routine).

7. Experimental Protocol

The following procedure was developed for use with the LightCycler® 1.x / 2.0 and 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 Prep. Kit').

Negative control: Always run at least one negative control - replace the template DNA with water.

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

7.1. Preparation of Parameter-Specific Reagents (PSR):

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

Check for the colored pellet, then **add 66 µl** PCR-grade water, mix (vortex) and spin down.

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

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

7.2. Preparation of the Reaction Mix

Include a Positive Control and at least one 'No Template Control' (NTC). In a cooled tube, prepare the reaction mix by multiplying the single reaction volumes by the number of reactions plus one reserve :

For use with the Roche FastStart Master	
Single reaction	**Component**
8.6 µl	water, PCR-grade (colorless cap, provided with the Roche Master kit)
2.4 µl	Mg^{2+} solution 25 mM (blue cap, provided with the Roche FastStart kit)
2.0 µl	**reagent** 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 2

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.

8. Data Analysis

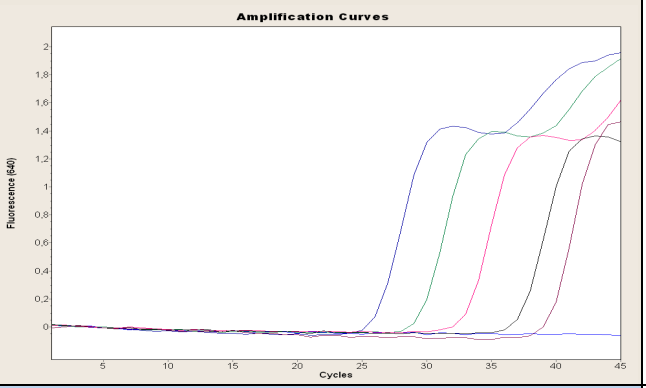
Perform data analysis, as described in the Instrument operator's manual.

We recommend using the Second Derivative Maximum method (Automated (F'' max)). The cycle number of the Crossing Point (Cp) of each sample is calculated automatically. The Fit Points method is more-error prone due to the user's influence.

View β-*Globin Extraction Control* data in channel 640, (LightCycler® 2.0 Instruments) or filter combination 483/498-640 (480 Instruments) Quantification mode.

The negative control (NTC) must show no signal.

8. Sample Data - Typical Results



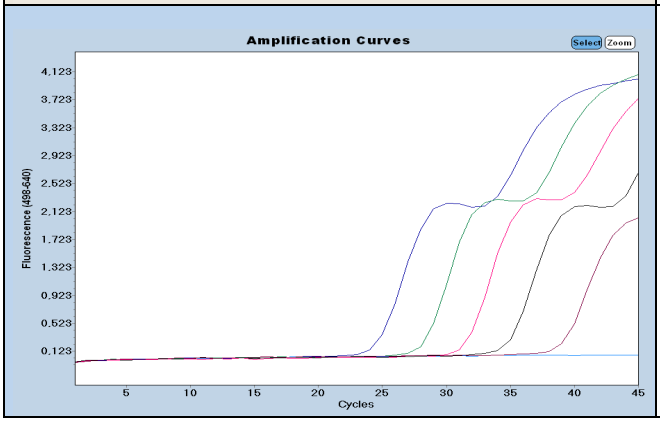
LightCycler® 2.0 Instrument

Roche Master: Fast Start

Sample data for *β-Globin Extraction Control*
Channel 640

Typical Cp-Values

100 ng hu. placenta DNA:	Cp 24-26
10 ng hu. placenta DNA:	Cp 27-29
1 ng hu. placenta DNA:	Cp 30-33
0.1 ng hu. placenta DNA:	Cp 34-36
0.01 ng hu. placenta DNA:	Cp 37-39



LightCycler® 480 Instruments

Roche Master: Fast Start

Sample data for *β-Globin Extraction Control*
Filter Combination 483-640 (498-640 version II)

Typical Cp-Values

100 ng hu. placenta DNA:	Cp 23-25
10 ng hu. placenta DNA:	Cp 26-28
1 ng hu. placenta DNA:	Cp 29-31
0.1 ng hu. placenta DNA:	Cp 32-34
0.01 ng hu. placenta DNA:	Cp 35-38

Fig.1. Sample data for the β-*Globin Extraction Control* detection system.

Upper panels: LightCycler® 2.0 Instrument. Left panel channel 640 quantification mode (Second Derivative Maximum)
Lower panels: LightCycler® 480 II Instrument. Left panel Filter combination 498- 640 quantification mode (Second Der Max)

9. Material Safety Data

According to OSHA 29CFR1910.1200, Australia [NOHSC:1005, 1008 (1999)] and the EU 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

V140212	Release version
V150303	Section 7. Experimental Protocol, use 2 µl (instead 4 µl).

Roche SAP order n° 07409168001

Notice to Purchaser

LightCycler® hybridization probes and kits produced under license agreements with Roche. The purchase price of this product includes a limited, nontransferable license under US patent claims and corresponding patent claims outside the United States, licensed from BioFire Defense, to use only this amount of the product for HybProbe assays and related processes described in said patents solely for the research and development activities of the purchaser. Diagnostic uses require a separate license from Roche.

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

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