Instructions For Use

LightMix® Modular Actin Extraction Control (ACTB) 660

Cat.-No. 66-0913-96 Roche SAP n° 07 805 993 001

Kit with reagents for 96 PCR reactions 20 µl for detection of the human Actin gene [lyophilized]

1. Content, Storage and Expiry

Storage at Arrival:

1 Vial green cap 96 reactions beta-Actin (lyophilized)

Store cooled or at ambient temperature
Do not freeze the lyophilized reagents.

- Lyophilized kits are stable for at least 6 months (4°C to 25°C in the dark). See lot-specific expiry date.
- Dissolved reagents are stable for at least 2 weeks if stored protected from light and cooled (4°C).
- Dissolved reagents can be stored long-term at -20°C (within expiry). Avoid multiple freeze-thaw cycles.

2. Additional Reagents required

LightCycler® Multiplex DNA Master Cat.-No. 07 339 585 001
or Roche LightCycler® 480 Probes Master (no instructions included) Cat.-No. 04 707 494 001

3. Introduction

PCR analysis of biological samples may occasionally result difficult due to problems related to the extraction process.False negative results can be due to the presence of inhibitors, degraded target material or an unsuccessful extraction of nucleic acids. The presence of amplifiable nucleic acids can be verified by running an extraction control PCR.

This product is intended to be used as extraction control and as reference gene for DNA-based assays and is compatible with KREC/TREC testing from newborn blood (53-0621-96 and 61-0622-96).

4. Description

A 83 bp long fragment from the intron 3 / exon 4 boundary of the human beta Actin gene (ACTB) is amplified with specific primers and detected with a LC670 labeled hydrolysis probe.

5. Specification

These reagents detect 0.01 ng or less of *vertebrate* DNA. The linear measuring range is 0.1 to 500 ng DNA.

6. Sample Material and Extraction

Depends on the analytical PCR. See ModularDx Document ***Extraction Protocols***.

7. Material Safety Data (MSDS)

According to OSHA 29CFR1910.1200, Australia [NOHSC:1005, 1008 (1999)] and the EU Directives 67/548/EC and 1999/45/EC any products which do 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.

8. Instructions for Use

• Instrument programming see document ***ModularDx Programming***
• Color Compensation see instructions in ***40-0320 Universal Color Compensation Hexaplex***
• Pipetting instructions multiplex PCR see ***ModularDx Multiplex***

8.1. Programming Roche 480 Instruments

See the Instrument operator's manual for details. Start programming before preparing the solutions. The protocol consists of four program steps:

• 1: Reverse Transcription of the viral RNA
• 2: Denaturation: sample denaturation and enzyme activation
• 3: Cycling: PCR-amplification
• 4: Cooling: cooling the instrument

Detection Format and Settings : **Set Quant Factor 10, Max Integration time 3 sec**
LightCycler® 480 Instrument: 615-670
LightCycler® 480 II Instrument: 618-660
cobas z 480 Analyzer (open channel): 610-670

Program Step:	***RT Step***	***Denaturation***	***Cycling***			***Cooling***
Parameter						
Analysis Mode	*None*	None	Quantification mode			None
Cycles	*1*	1	45			1
Target [°C]	55	95	95	60	72	40
Hold [hh:mm:ss]	00:05:00	00:05:00	00:00:05	00:00:15	00:00:15	00:00:30
Ramp Rate [°C/s] **96**	4.4	4.4	4.4	2.2	4.4	1.5
Ramp Rate [°C/s] **384**	4.6	4.6	4.6	2.4	4.6	2.0
Acquisition Mode	None	None	None	**Single**	None	None

** 1-Step RT-PCR optional* Table 1

8.2. Experimental Protocol

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

For an increased sensitivity use 10 µl sample per 20 µl reaction, in case that inhibition is likely to occur, e.g. extracts obtained from fecal samples, use 5 µl. For 10 µl reactions in 384 well plates use 5 µl /2.5 µl.

8.2.1. Preparation of Parameter-Specific Reagents (PSR, 96 reactions):

One reagent vial with a **green** cap contains all primers and probe to run 96+ LightCycler® reactions.

Add 50 µl PCR-grade water to each reagent vial, mix the solution (vortex) and spin down.
For robotic pipetting the volume can be extended to 55 µl (signals will decrease by 10-20%).

►**Use 0.5 µl** reagent for a 20 µl PCR reaction.

8.2.2. Preparation of the Target DNA

Actin is a control reaction working with genomic DNA. Follow the procedure for the analytical PCR.

8.2.3. Preparation of the Reaction Mix Multiplex DNA Master

In a cooled reaction tube, prepare the reaction mix for single reactions (left) or one plate (right). The table contains the amounts for a duplex reaction for one analytical parameter (red) and the control:

Duplex PCR - Instruction for use with the Roche LightCycler® Multiplex DNA Master		
5 µl extract	**Component**	**10 µl extract**
10.0 µl	**Water**, PCR-grade (colorless cap, provided with the Roche Master kit)	5.0 µl
0.5 µl	**Actin Control Reaction** and	0.5 µl
0.5 µl	**Reagent** mix additional assay(s) (Multiplex PCR))	0.5 µl
4.0 µl	**Roche Master** (see Roche manual)	4.0 µl
15.0 µl	**Volume of Reaction Mix** (DNA Master 14.9 µl)	**10.0 µl**

Table 2

Mix gently, spin down and **transfer 15 µl (10 µl)** per well.

Add 5 µl (10 µl) of sample or control to each well for a final reaction volume of 20 µl. Seal plate and centrifuge.

Start run

9. Typical Results (Data from LightCycler® 480 II system)

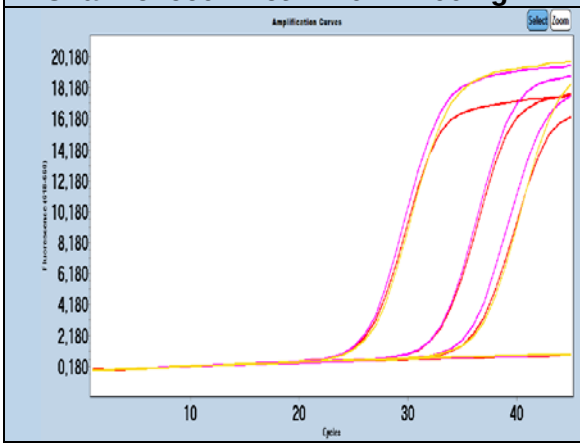
Channel 660 : Actin – 0.1 - 100 ng DNA	Data obtained in Multiplex PCR
	

Figure 1

10. Reading the Results

Perform data analysis as described in the operator's manual. For multiplex assays select the color compensation. We recommend using the Second Derivative Maximum method (Automated (F" max). View results in the 660 channel. The negative control (NTC) and negative samples must show a signal.

Analytical PCR (sample)	Channel 660 Control Reaction	Analytical PCR NTC Control	Result
No amplification	**Amplification** +	Negative	Parameter - Negative
Amplification+	Not relevant	Negative	Parameter- Positive
No amplification	**Not detectable**	Not relevant	**PCR failure Repeat**
Amplification signal	Not relevant	Positive	Contamination Repeat

Notes: cobas z 480 Analyzer signal levels are about 50% compared to LightCycler® 480 II results.
+ Cp depends on the respective dilution during extraction copies.

11. References

Neonatal screening for SCID using high-throughput triplex R PCR. Borte et al., 2012

12. Multiplex PCR Compatibility

The Actin extraction control / reference assay can be combined with up to three (LightCycler® 96), four (cobas z 480) or five (LightCycler® 480 system) analytical assays :

Multiplex PCR and Instrument Compatibility

Color Compensation 40-0320 is mandatory for Multiplex PCR

500	FAM	580	610	640	660	480 II	z 480	LC96	LC2.0	Nano
	Assay 1				Actin	X	X	X		
	TREC		KREC		Actin	X	X	X		
	Assay 1	Assay 2	Assay 3		Actin	X	X	X		
	Assay 1	Assay 2	Assay 3	Assay 4	Actin	X	X			
Assay 5	Assay 1	Assay 2	Assay 3	Assay 4	Actin	X				

Table 3

13. Version History

V151001	2015 protocol Multiplex Master, 5/10 µl extract and 60°C acquisition	2015-10-01

Certificate of Analysis (CoA)

Lot n°
Expiry :

Dilution	**-**	**-**	**100 ng**	**1 ng**	**0.1 ng**	**-**	**passed**
Cp range Measured				32-34			
Signal level Measured				10-20			
Negatives	**10/10**						

***Note**:* Cp (crossing point) values collected with pDNA (single target PCR). Fluorescence (FL) levels depend on instrument settings and may vary. The Cp values will vary from instrument to instrument by up to 2 cycles, while the distance between two dilution steps should be relative constant (ΔCp).

QC Acceptance Date: YYYYMMDD

We, the undersigned, certify that the product designated above has been obtained in accordance with the rules of production and quality control.

Name(s) :

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