Instructions For Use

LightMix® Modular MSTN Extraction Control 660

Cat.-No. 66-0905-96 Roche SAP n° 07 225 253 001

Kit with reagents for 96 PCR reactions 20 µl for detection of MSTN [lyophilized]

1. Content, Storage and Expiry

Storage at Arrival:

1 Vial green cap 96 reactions MSTN (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 RNA Virus Master	Cat.-No. 06 754 155 001
or 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. In particular, false negative results in pathogen testing 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 or RNA-based assays. The targeted region of the Myostatin (MSTN) gene is highly conserved in chordata.

4. Description

A 89 bp long fragment from the myostatin gene or transcript is amplified with specific primers and detected with a LC670 labeled hydrolysis probe. Starting with lot 3370 this kit has been improved by adding one primer and one probe to detect a wider variety of species.

5. Specification

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

The product has been confirmed to work with samples from human (*Homo sapiens*), porc (*Sus suis*), beef (*bos bovis*), dromedary (Camelus dromedarius), chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*) ostrich (*Struthio camelus*), mallard (*Anas platyrhynchos*), pigeon (*Columba livia*), quail (*Coturnix coturnix*) guinea fowl (*Numida meleagris*), duck muscovy (*Cairina moschata*), and goose (*Anser ansermallard*).

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 660 Channel **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.

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 Nucleic Acids

MSTN is a control reaction working with DNA and RNA. Follow the procedure for the analytical PCR.

8.2.3. Preparation of the Reaction Mix RNA/DNA Multiplex Master

Multiply volumes by the number of reactions plus controls and one reserve and prepare in a cooled tube. 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 RNA Virus Master		
for 5 µl extract	**Component**	**10 µl extract**
9.9 µl	**Water**, PCR-grade (colorless cap, provided with the Roche Master kit)	4.9 µl
0.5 µl	**MSTN 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
0.1 µl	**RT Enzyme** (RNA Master only)	0.1 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)

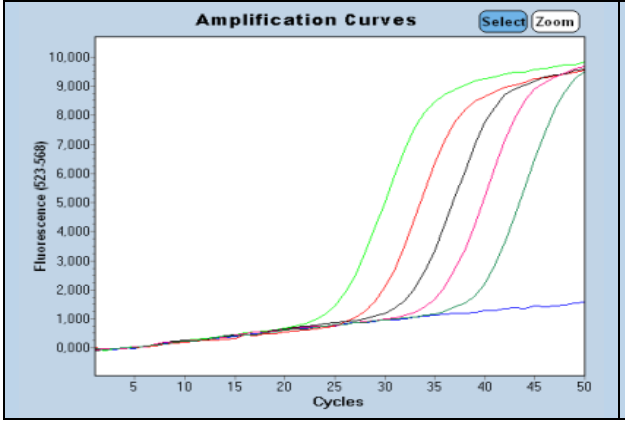
Channel 660 : MSTN – Single PCR	Data obtained in Multiplex PCR
	

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

Analytical Performance Determination and Clinical Validation of the Novel Roche RealTime Ready Influenza A/H1N1 Detection Set. Wenzel et al. (2010)

12. Multiplex PCR Compatibility

The MSTN 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	530	580	610	640	660	480 II	z 480	LC96	LC2.0	Nano
	Assay 1				MSTN	X	X	X		
	Assay 1	Assay 2	Assay 3		MSTN	X	X	X		
	Assay 1	Assay 2	Assay 3	Assay 4	MSTN	X	X			
Assay 5	Assay 1	Assay 2	Assay 3	Assay 4	MSTN	X				

Table 3

13. Version History

V140404	Release version	2014-04-14
V150404	LightCycler® Nano removed from allowed instruments	2015-04-10
V150525	Sample volume, instrument settings and PCR cycles (Multiplex Master) One probe added - spectrum of detected (bird) species enlarged	2015-05-25
V151001	2015 protocol Multiplex Master, 5/10 µl extract and 60°C acquisition	2015-10-01
V160313	8.2.3 wording	2016-07-07

Certificate of Analysis (CoA)

Lot n°
Expiry :

Dilution	Placenta DNA	100 ng	10 ng	1 ng	passed
Cp range		23-26	26-29	30-33	
Measured					
Signal level		5-15			
Measured					
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) :

TIB MOLBIOL Syntheselabor GmbH | Eresburgstr. 22-23 | D-12103 Berlin | Germany
Tel. +49 30 78 79 94 55 | FAX +49 78 79 94 99 | dna@tib-molbiol.de | WWW.TIB-MOLBIOL.COM
Geschäftsführer (CEO): Olfert Landt | Register HRB 93163 B | Registergericht Berlin Charlottenburg