



Manual for Meat-PCR-Kit

I. Description

The Meat-PCR is a PCR System for the LightCycler to quantify Bovine, Pork and Mammalian (Myostatin gene) DNA

The following specifications can be achieved:

Detection limit: 0.01%

Working range for the quantification above 0.1%

Variation of results: 30% (relative to the standard)

Uncertainty: +5% -5%

II. Product Components

Material enclosed by the Kit :

■ **MasterMix for Bovine** (yellow tube)

■ **MasterMix for Pork** (green tube)

■ **MasterMix for Myo** (red tube)

■ **iSt BoPoMo** (transparent tube), calibrated against a mixture of DNA of Bovine meat and vegetarian Animal Feed

Storage Conditions:

-20°C at least 6 months,

Never refreeze the components,

To **avoid contamination** store the Standard (iSt BoPoMo) separated from the other components!

Material NOT enclosed by the Kit :

QuantiTect SYBR Green PCR Kit 200rxnNr. 204143 from www.Qiagen.com (Taq Polymerase)

Water (PCR-grade,) = sH₂O

Tubes (RNase, DNase free)

LightCycler, Taqman, iCycler or other real-time PCR instrument

III. Additional Components

Available for free on request, delivered as attachment (e-mail) and/or diskettes

■ **Setup Sheet:** EXCEL file for PC and Macintosh

■ **Calculation Sheet:** EXCEL file for PC and Macintosh

■ **Experiment File:** Thermal cycling program to be used with the LightCycler Program. Copy the files into the Protocol folder of the LightCycler Programme before use

■ **Sample Data Entry File:** To be used with the LightCycler Program. Copy the files into the Protocol folder of the Lightcycler Program before use

IV. Analysing Strategy

Use different solutions for the template (sample DNA). For example 3 dilutions: 1:20, 1:40, 1:80 of template DNA (photometrically determined) per assay (do not exceed 400ng per assay).

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V. Ordering information

Product	Size	Cat.#
Meat-PCR Kit For Research Use Only. Not for use in diagnostic procedures.	96 Assays	22005

VI. Reaction Protocol

Preparations

Store the components at +4°C for one hour for completely thawing and homogenization.

Amplification Reactions *(use Setup Sheet, see Chapter III.)*

Component	Single Reactions	12 Reactions (+5%)
MasterMix Bov Pork or Myo	5µl	63µl
Quantitect Master Mix	10µl	126µl
Mix gently (no vortex!)		
Distribute to each capillary	15µl	15µl
add sample DNA (160ng/10µl) or standard	5µl	5µl
Total per capillary	20µl	20µl
Seal each capillary with a stopper centrifugate at 700 x g for 5 sec (3000rpm in a standard benchtop microcentrifuge)		

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Thermal Cycling Programme

PROGRAM	DENAT		PCR				MC			COOL	
	Cycles	Analysis Mode	Cycles	Analysis Mode			Cycles	Analysis Mode		Cycles	Analysis Mode
Anwendung Bsp.	Target Temp.		Target Temp.				Target Temp.			Target Temp.	
	Incub. Time		Incub. Time				Incub. Time			Incub. Time	
	Trans. Rate		Trans. Rate				Trans. Rate			Trans. Rate	
	Sec.Target Temp.		Sec.Target Temp.				Sec.Target Temp.			Sec.Target Temp.	
	Step Size		Step Size				Step Size			Step Size	
	Step Delay (Cycl)		Step Delay (Cycl)				Step Delay (Cycl)			Step Delay (Cycl)	
	Acquisition Mode		Acquisition Mode				Acquisition Mode			Acquisition Mode	
Pork	1	None	45	Quant			1	MC		1	None
	95		95	66	72	72	95	50	95	40	
	15:00		10	20	20	2	5	5	0	10	
	20		20	20	20	20	10	3	0.2	20	
	0		0	0	0	0	0	0	0	0	
	0		0	0	0	0	0	0	0	0	
	0		0	0	0	0	0	0	0	0	
	None		None	None	None	Single	None	None	Cont	None	
Bovine and Myostatin	1	None	43	Quant			1	MC		1	None
	95		95	56	72	72	95	50	95	40	
	15:00		10	20	20	2	5	5	0	10	
	20		20	20	20	20	10	3	0.2	20	
	0		0	0	0	0	0	0	0	0	
	0		0	0	0	0	0	0	0	0	
	0		0	0	0	0	0	0	0	0	
	None		None	None	None	Single	None	None	Cont	None	

Edit samples: use **Sample Data Entry File**, see Chapter III

Data interpretation

Chanel

F1: Signal for Pork, Bovine and Myostatin

Use the second derivative method for the quantification

Fill in the **Calculation Sheet** (see Chapter III.) with data from the LightCycler. Use results which behave approximately proportional to the dilution of the sample.

^(a) For Research Use Only. Not for use in diagnostic procedures.

^(b) Biosmart GmbH, Switzerland, developed the quantification system Duplex Lectin-35S. Modifications are reserved.

^(c) The PCR method stays under US Patent owned by Roche, Inc. The application of PCR requires a license. Nothing of this publication may be understood as a warrant or as a license for applying the PCR method.

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All prices and specifications are subject to change without prior notice.
 Berne, 9 August 2001