



# **REAL-TIME PCR**



TaqMan<sup>®</sup> Detection & Multiplex – SYBR<sup>®</sup> Green Detection Direct Amplification – Lyophilisation – Supplements Dual Labeled Probes

Molecular Biology 

### Unleash the Power of Real-Time PCR with ready-to-use reagents, customized solutions and comprehensive scientific support!

Master mixes – Freeze-dried reagents – OEM & Bulk supplier

# WE DEVELOP LIFE SCIENCE REAGENTS

Jena Bioscience, with over 25 years of experience in academic know-how, is a leading provider of innovative and high-quality reagents and customized services in the life science field.

We have successfully served clients in over 100 countries, offering tailored solutions for DNA and RNA amplification.

Our extensive portfolio includes single reagents, complex kits, and optimized master mixes for purification, amplification, and modification of DNA.

We provide reliable and efficient solutions for all your PCR-related techniques.

### Certification – Ensuring Quality and Excellence

Our state-of-the-art production facility and the comprehensive quality management system in accordance with DIN EN ISO 9001 and DIN EN ISO 14001 ensures highest quality standards for all our products.



IFTA AG Certified QMS and EMS according to DIN EN ISO 9001 and DIN EN ISO 14001 Reg.-No.: ICV03597 034 and ICV03597 534







# REAL-TIME PCR PRODUCTS

Unmatched price structure to performance ratio in the market



#### Lab to Bulk

Move from lab to bulk scale for more flexibility with variable quantity options.

#### Liquid & Lyophilized

Convenience and ease for use of liquid products and long time storage at room temperature with lyophilized options.

#### Ready-to-use & customized

Tailored RT-qPCR products, custom-made to match your exact research requirements.





		Direct amplification				
SYBR®Green	•			•		
TaqMan®		•	۰	•		
Specificity	•	• •	• •	• •		
Sensitivity	• <sup>1</sup>	• •	• •	• •		
Multiplexing		•	• •	• •		
Costs	•	• •	• • •	• •		
Turnaround time	•	•	٠	• •		
ligh throughput •		•	• • •			
		Applicatio	ns			
Genexpression	•	•	٠	٠		
Genotyping	•	•	۲	•		
Mutation detection		•	٠	•		
Pathogen identification		•	٥	•		
Species diversity analysis	•	• •	• •	• •		

<sup>1</sup> variable





# SYBR<sup>®</sup>Green REAL-TIME PCR

Dye based with high throughput potential

The **fluorescence dye SYBR®Green** intercalates into double-stranded DNA molecules during PCR reaction.

PCR mixture with template DNA, primers and fluorescence dye (SYBR®Green) is heated to denature the DNA strands.

DNA polymerase prolongs the DNA. SYBR®Green intercalates specifically into double stranded DNA and the fluorescence intensity increases in direct correlation with the amount of DNA present.

### Method of SYBR®Green Real-Time PCR





### Functional QC: Amplification of human DNA and E. coli DNA templates

Reproducible and low variability levels with various starting DNA amounts. Amplification plot of ß-actin gene from human DNA and of 16S rRNA gene for detection of bacterial DNA (E.coli). qPCR SybrMaster #PCR-374 was used for real-time PCR. Starting DNA amount was 1pg, 10 pg and 100 pg of E. coli and 10 pg, 100 pg and 1 ng of human DNA.







CatNo.	Amount	Conc.	Reactions
qPCR SybrMaster			
PCR-372S	2 × 1.25 ml	2×	250 reactions×20μl
PCR-372L	10 × 1.25 ml	2×	1,250 reactions×20μl
qPCR SybrMaster lowROX			
PCR-373S	2 × 1.25 ml	2×	250 reactions×20μl
PCR-373L	10 × 1.25 ml	2×	1,250 reactions×20μl
qPCR SybrMaster highROX			
PCR-374S	2 × 1.25 ml	2×	250 reactions×20μl
PCR-374L	10 × 1.25 ml	2×	1,250 reactions×20μl
qPCR SybrMaster UNG			
PCR-375S	2 × 1.25 ml	2×	250 reactions×20μl
PCR-375L	10 × 1.25 ml	2×	1,250 reactions×20μl



### Did you know? - UNG

Uracil-N-Glycosylase specifically cuts uracil from DNA by cleaving the glycosidic bond. It is used to decontaminate PCR assays from previous amplified DNA. The PCR reaction is performed with dUTP instead of dTTP. Before the next run is started, UNG incubation (followed by heat inactivation) prevents potential carry-over contamination.



# TaqMan<sup>®</sup> REAL-TIME PCR

Probe based with high sensitivity and specificity

Specially designed **TaqMan® probes** bind to the target sequence and emit a signal when amplified.

PCR mixture with template DNA, primers and TaqMan® probes labeled with a reporter dye (R) and a quencher (Q) is heated to denature the DNA strands.

DNA polymerase prolongs the DNA strand and the exonuclease activity degrades the probe. As the reporter dye is no longer in close proximity to the quencher, the resulting increase in reporter emission intensity is easily detected.

#### TaqMan<sup>®</sup> ProbesMaster Kits

- → Contain all reagents
  (just add template, primer and probes)
- → Hot-start polymerase
- → With or without UNG (Uracil-N-Glycosylase)
- → No ROX, low ROX or high ROX as reference dye for cycler-internal signal normalization

### Method of TaqMan® Real-Time PCR







CatNo.	Amount	Conc.	Reactions		
qPCR ProbesMaster					
PCR-360S	2 × 1.25 ml	2×	250 reactions×20μl		
PCR-360L	10 × 1.25 ml	2×	1,250 reactions×20µl		
qPCR ProbesMaster lowROX					
PCR-361S	2×1.25 ml	2×	250 reactions×20μl		
PCR-361L	10 × 1.25 ml	2×	1,250 reactions×20µl		
qPCR ProbesMaster highROX					
PCR-362S	2 × 1.25 ml	2×	250 reactions×20μl		
PCR-362L	10 × 1.25 ml	2×	1,250 reactions×20µl		
qPCR ProbesMaster UNG					
PCR-363S	2 × 1.25 ml	2×	250 reactions×20μl		
PCR-363L	10 × 1.25 ml	2×	1,250 reactions×20µl		
qPCR ProbesMaster UNG lowROX					
PCR-364S	2 × 1.25 ml	2×	250 reactions×20μl		
PCR-364L	10 × 1.25 ml	2×	1,250 reactions×20μl		
qPCR ProbesMaster UNG highROX					
PCR-365S	2 × 1.25 ml	2×	250 reactions×20μl		
PCR-365L	10 × 1.25 ml	2×	1,250 reactions × 20 μl		



### Did you know? - ROX

The reference dye normalizes fluctuations of fluorescence signal caused by the PCR cycler or pipetting differences. ROX does not affect the PCR reaction but maintains a stable fluorescence baseline. The use of ROX (no/low/high) depends on the cycler type, which should be checked in the operating manual.



# TaqMan<sup>®</sup> MULTIPLEX PCR

Amplification of multiple targets in a single tube

- → Simultaneous real-time analysis of > 2 target sequences
- → Robustness against a multitude of PCR inhibitors
- → Excellent sensitivity for amplification of lowest template amounts

### PCR with all four primer pairs from a single tube





#### **Meat Detection and Food Control**

4-plex reaction using the qPCR MultiplexMaster #PCR-340. Amplification plot for 4-plex reaction with 1ng DNA of various meat samples (pork, horse, beef) as well as an internal positive control.







CatNo.	Amount	Conc.	Reactions
qPCR MultiplexMaster			
PCR-340S	2 × 1.25 ml	2×	250 reactions×20μl
PCR-340L	10 × 1.25 ml	2×	1,250 reactions×20µl



### Did you know? – Multiplex Direct Amplification

Our MultiplexMaster Kit can also be used for direct amplification without prior DNA purification by adding our Extraction Buffer #PCR-534 to the sample. More info: pp 12 and 16



# DIRECT AMPLIFICATION

No need for time-consuming DNA extraction - perfect for Point-of-Care applications

#### Pro

- → Automatable for high throughput
- → Reduce DNA preparation time by 70-90%
- → No inhibition for a multitude of sample matrixes
- → Time & cost efficient
- → Minimize sample loss
- → Less plastic consumables

#### Contra

- → Complex matrixes can interfere with PCR
- → Lower sensitivity





Comparison of classical DNA purification prior to amplification and direct amplification with different sample matrices (left figure: human saliva sample right figure: pork meat sample)

Amplification of ß-actin gene. Sample DNA was prepared with **Tissue DNA Preparation – Column Kit #PP-236**.

Direct amplification was performed without purification. **Direct qPCR ProbesMaster #PCR-396** (human saliva) and **MeatDetect qPCR Pork (Halal) #PCR-701** (pork) were used for real-time PCR.



### Method of Direct Amplification





CatNo.	Amount	Conc.	Reactions
Direct qPCR SybrMaster			
PCR-344S	2 × 1.25 ml	2×	250 reactions×20μl
PCR-344L	10 × 1.25 ml	2×	1,250 reactions×20μl
Direct qPCR SybrMaster highROX			
PCR-345S	2 × 1.25 ml	2×	250 reactions×20μl
PCR-345L	10 × 1.25 ml	2×	1,250 reactions×20μl
Direct qPCR ProbesMaster			
PCR-396S	2 × 1.25 ml	2×	250 reactions×20μl
PCR-396L	10 × 1.25 ml	2×	1,250 reactions×20μl
Direct qPCR ProbesMaster highROX			
PCR-397S	2 × 1.25 ml	2×	250 reactions×20μl
PCR-397L	10 × 1.25 ml	2×	1,250 reactions×20μl



### LYOPHILISATES

Long term storage without cooling

### → Ready-to-use reagents: Pre-aliquoted with all required reagents

- → No fridge required
- → Stable at ambient temperature
- → Reduced contamination risk



### Lyophilisation Brochure

Have a look at our lyophilisation brochure. Feel free to request your copy: **molbio@jenabioscience.com** 

### **Lyophilisation Service**

Tailored lyophilisates according to your requirements. Contact us for customized lyophilisation services: **molbio@jenabioscience.com** 









### **Lyophilized Mixes**

Ready-to-use lyophilisates with a complete master mix<sup>1</sup> in a dry, room temperature stable format

CatNo.	Reactions
qPCR SybrMaster Lyophilis	ate
PCR-173S	192 reactions × 20 μl
PCR-173L	960 reactions × 20 μl
qPCR ProbesMaster Lyoph	lisate
PCR-156S	192 reactions × 20 μl
PCR-156L	960 reactions × 20 μl

 $^{1}\mbox{Just}$  add template DNA in water and start the cycler.

### **Liquid Mixes for Lyophilisation**

Liquid master mixes optimized for freeze-drying in your own production facilities

CatNo.	Amount	Conc.	Reactions
qPCR SybrMaster Lyophilisate			
PCR-189-1ML	1 ml	2.5 ×	125 reactions × 20 µl
PCR-189-10ML	10 ml	2.5 ×	1,250 reactions×20µl
qPCR ProbesMaster Lyophilisate			
PCR-188-1ML	1 ml	2.5 ×	125 reactions × 20 µl
PCR-188-10ML	10 ml	2.5 ×	1,250 reactions×20μl



### **SUPPLEMENTS**

**Helpful tools** for real-time PCR functional testing or quality control of qPCR polymerases and qPCR assay conditions.

- → Thermolabile UNG
- → DNA Stain
- → Reference Dye
- → Direct Extraction Buffer





CatNo.	Amount	Conc.					
Thermolabile UNG (Uracil N-Glycosylase)							
PCR-353	200 units	1 unit/µl					
SYBR <sup>®</sup> Green Fluorescent I	DNA Stain						
PCR-378	500 µl	100 µM					
ROX Reference Dye							
PCR-351	1ml	25 µM					
PCR-356-1ML	1ml	100 µM					
Direct Extraction Buffer							
PCR-534-15ML	15 ml	10 ×					
PCR-534-100ML	100 ml	10 ×					



# **DUAL LABELED PROBES**

**DNA oligonucleotides** carrying a fluorophore (5'-end) and a quencher (3'-end). The labeled probe hybridizes sequence-specifically to its complementary sequence on the amplicon.

### $\rightarrow$ Increase efficiency and specificity

- → Enable multiplex analyses
- → Maximal assay design flexibility

### Probes and quencher are available in the following concentrations:

- → 5 to 9 nmol
- → 10 to 19 nmol
- $\rightarrow$  20 to 29 nmol
- → 30 to 49 nmol
- → 50 to 70 nmol

- → **Purification:** HPLC
- → Quality check: MALDI TOF
- → Sequence lengths: up to 40 bp
- → **Customized combinations** available

#### Selecting the correct reporter dye and quencher

Criterion	Reporter Dye Selection Quencher Selection				
Instrument compatibility	Ensure compatibility with your real-time PCR instrument's detection channels				
Background signal	Ensure low background signal for accura	ite measurements			
Spectral characteristics	Choose reporter dyes with minimal spectral overlap for multiplexing	Dark quencher are often preferred for multiplexing			
Fluorescence emission maximum (λ <sub>max</sub> )	Match reporter dye's $\lambda_{max}$ with your instrument's filter settings				



# SELECTING REPORTER DYE AND QUENCHER

Select from Jena Bioscience's extensive reporter/quencher repertoire or inquire for alternative combinations: **molbio@jenabioscience.com** 

			3'-Quencher							
5	' Reporter			BHQ-1®	BHQ-2®	BHQ-3®	BHQ-650	ECLIPSE	DABYL	TAMRA*
	Excitation	Emission	Quenching rate [nm]	480-580	550-650	620-730	550-750	390-625	380-550	470-560
	max [nm]	max [nm]	Quenching max. [nm]	535	579	672	650	522	453	544
ATTO-390	390	476							•	•
ATTO-425	439	485						•	•	•
LC*Cyan500	450	500						•	•	
6-FAM	495	520		•	•			•	•	•
Fluo	495	520		•	٠			•	٠	•
FITC	490	525		•	٠			•	٠	•
ATTO-495	498	526		•				•	٠	•
TET	521	536		•	•			•	•	•
ATTO-520	517	538		•				•	٠	•
JOE	522	548		•				•	٠	•
Yakima Yellow	530	549						•	•	•
HEX	535	556			•		•	•	٠	•
ATTO-Rho6G	533	557		0	٠		٠	۰	٠	
СуЗ	546	563			٠		٠	۰		
TAMRA'	564	579			•		٠	٠	•	
ROX	576	601			٠		•	•		٠
Texas Red	586	610			٠		٠	۰		
LC*Red610	590	610			٠		٠	۰		
ATTO-Rho13	603	627			٠	٠	٠			
DY480ßXL	500	630			•		•			
LC'Red640	625	640			٠	•	٠			
ATTO-Rho14	625	646	-		٠	•	٠			
CY5	646	662			٠	•	٠			
CY5.5	683	705			٠	•	٠			•
IRD700	685	705			•	•	•			•



### Did you know? - Black Hole quencher and TAMRA

Black Hole dark quencher (BHQ) probes are designed to absorb the probe fluorescence almost completely, thus ensuring a very high signal-to-noise ratio. Nevertheless, TAMRA is still used as quencher, especially in combination with the reporter dye FAM. Please note that TAMRA is not a dark quencher and contributes to an increase in the background signal due to its own fluorescence emission.



### **Contact our Real-Time PCR experts**

Send us an e-mail: molbio@jenabioscience.com



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