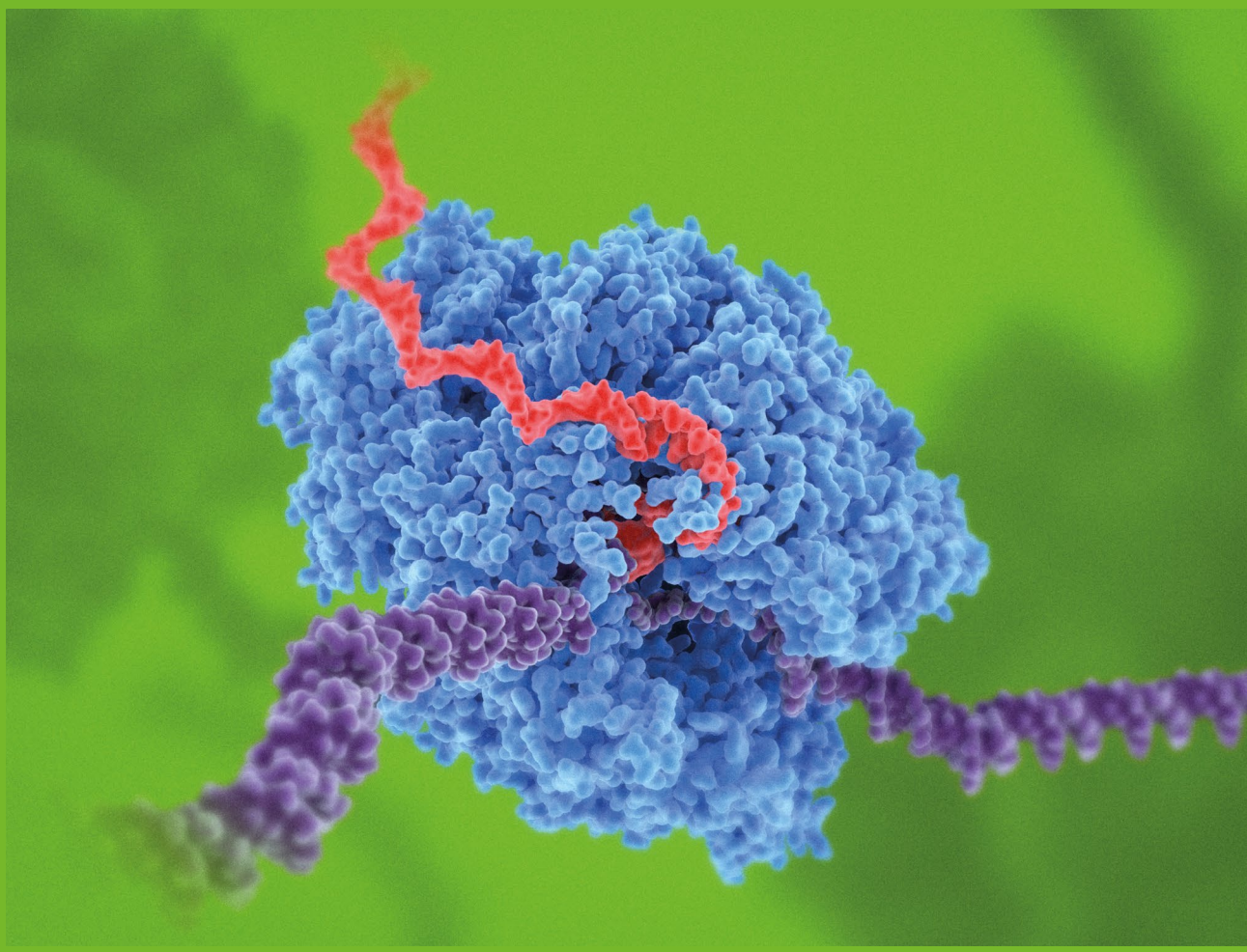
Jena Bioscience

Stratech

REVERSE TRANSCRIPTION REAL-TIME PCR



SYBR® Green & TaqMan® Master Mixes
Direct Amplification – Lyophilisates
Classics & Components

Molecular Biology

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

REVERSE TRANSCRIPTION REAL-TIME PCR PRODUCTS

Unmatched price structure to performance ratio in the market

Reverse Transcription qPCR Master Mixes

Ready-to-use solutions

- SYBR®Green Detection
- TaqMan® Detection
- Direct Amplification

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Lyophilisates

Room temperature stable

- SYBR®Green Detection
- TaqMan® Detection
- Customization service

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Components and Classics

Single reagents in bulk scale

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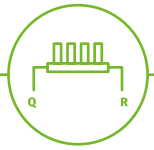


Dual-labeled Probes

Quencher and Oligos

Page 22

Lab to Bulk

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

Liquid & Lyophilized

Convenience and easy handling of liquid products and long-term storage at room temperature with lyophilized options.

Ready-to-use & customized

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

REVERSE TRANSCRIPTION

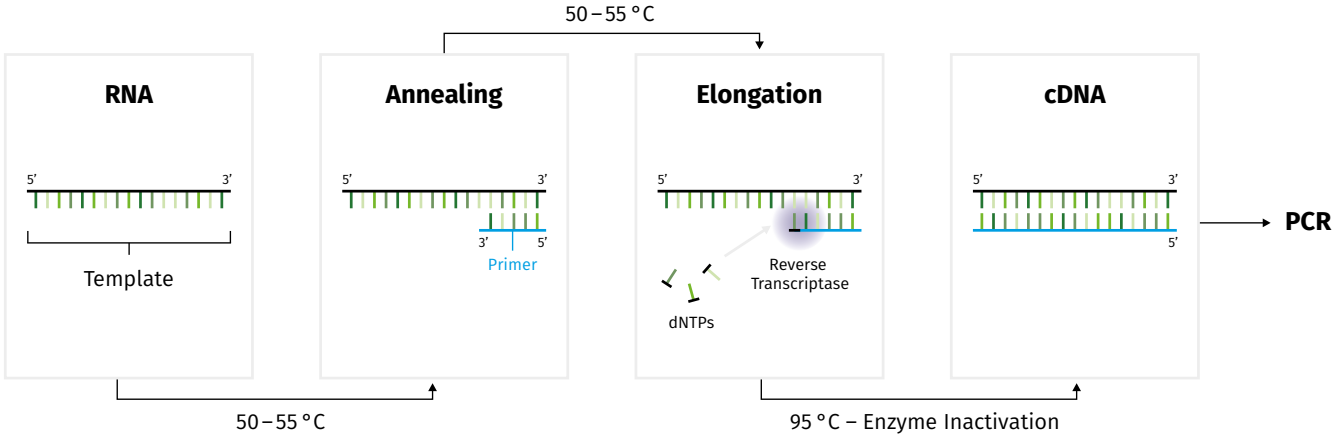
Turning RNA into utilizable and stable DNA

In **reverse transcription**, DNA is synthesized from RNA template using reverse transcriptases (RTs), creating complementary DNA (cDNA).

Applications:

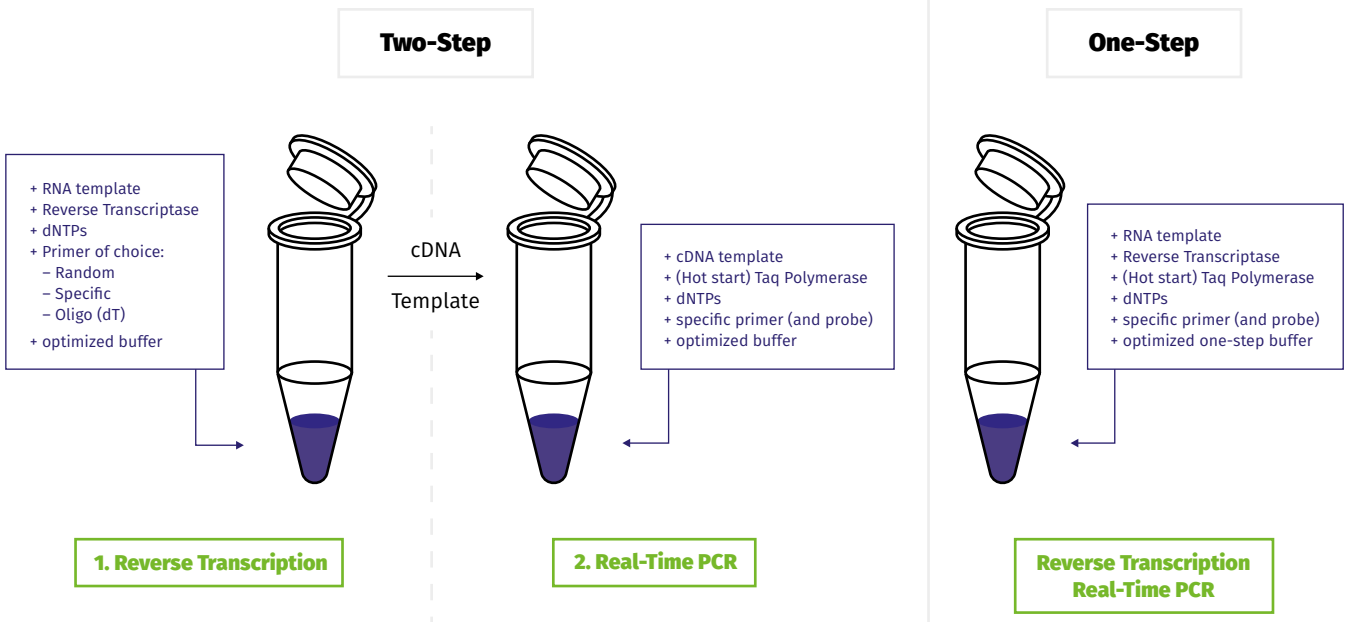
- PCR & Real-Time PCR (qPCR)
- Gene expression analysis
- Sequencing
- Probe labeling
- Cloning
- Viral load measurement

Method of Reverse Transcription



Reverse transcription starts with a single-stranded RNA template and primers that bind at the corresponding sites. The reverse transcriptase then binds to the RNA and generates the cDNA. Once the cDNA is synthesized, (q)PCR can be performed.

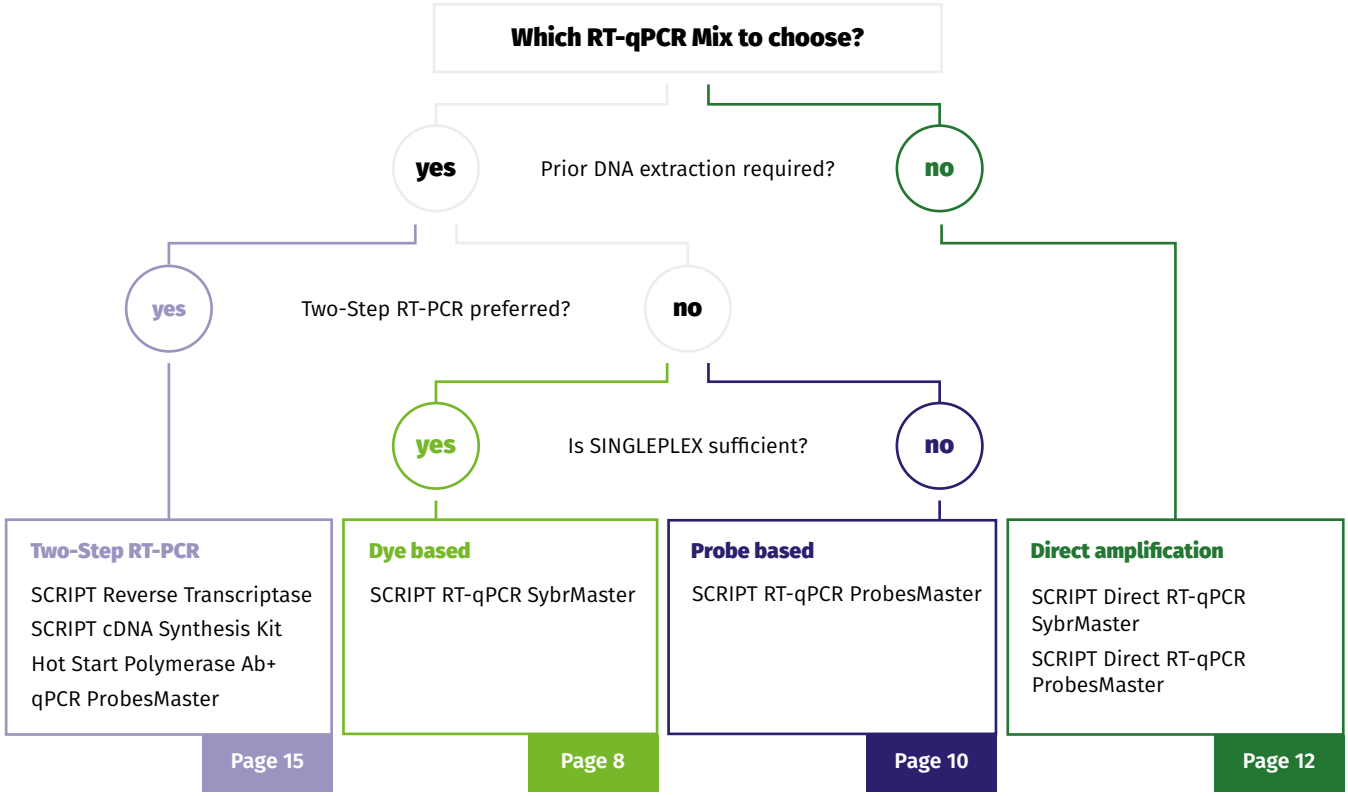
TWO-STEP VS. ONE-STEP qPCR REVERSE TRANSCRIPTION



	Two-Step	One-Step
Steps	Two separate reactions	One combined reaction
Primers	Oligo(dT), hexamers, octamers, gene specific	Gene specific
High throughput		•
Speed	•	• •
cDNA storage	•	
Sensitivity	• •	•
Risk of Contamination	• •	•

All in One: Our master mixes for Reverse Transcription Real-time PCR (RT-qPCR) are based on the one-step technology. For two-step RT-qPCR, we offer the following products:

- → **SCRIPT cDNA Synthesis Kit** #PCR-511 – Page 15
- → **qPCR ProbesMaster** #PCR-360
- → **qPCR SybrMaster** #PCR-372

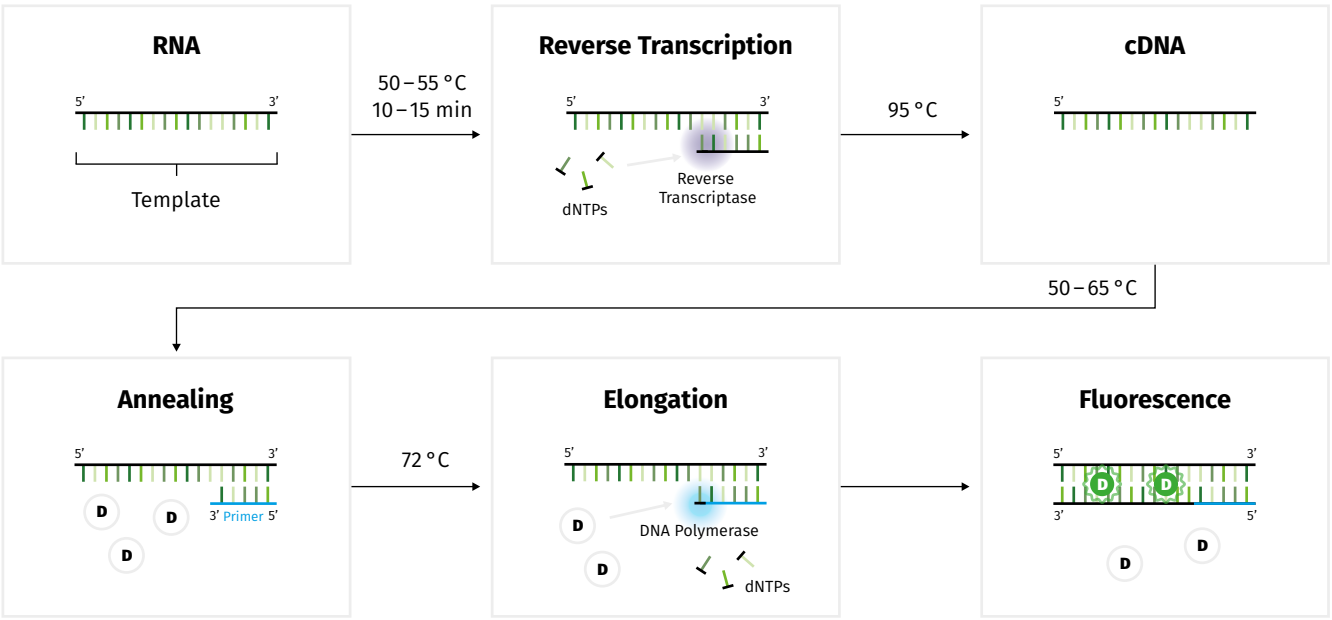


	Reverse Transcription qPCR		
	Extraction based amplification		Direct amplification
SYBR®Green	•		•
TaqMan®		•	•
Specificity	•	• •	• •
Sensitivity	•[1]	• •	• •
Multiplexing		•	• •
Costs	•	• •	• •
Turnaround time	• •	•	• •
High throughput	•	•	• •
	Applications		
Genexpression	•	•	•
Genotyping	•	•	•
Mutation detection		•	•
Pathogen identification		•	•
Species diversity analysis	•	• •	• •

[1] variable

SYBR®Green RT-qPCR

Dye based with high throughput potential



In a first step, the RT-qPCR mixture with reverse transcriptase, DNA polymerase, dNTPs and the fluorescent dye SYBR®Green generates cDNA from an RNA template and a corresponding gene-specific primer.

In the second step, the cDNA is amplified by PCR and SYBR®Green intercalates specifically into double-stranded DNA. The fluorescence intensity increases in direct correlation with the amount of DNA present.

Did you know? – Extreme Thermolabile UNG

Uracil-N-Glycosylase is used to prevent carry-over contamination with dU-containing DNA from previous reactions prior to the reverse transcription step. It catalyzes the hydrolysis of the N-glycosidic bond between the uracil and sugar, leaving an apyrimidinic site in uracil-containing single or double-stranded DNA. Extreme Thermolabile UNG is fully active at temperatures between 15 °C to 25 °C and inactivated at 50 °C.

SCRIPT RT-qPCR SYBRMASTER KITS

Features:

- All reagents included (just add template and primer)
- Reverse transcriptase with enhanced thermal stability
- Antibody-blocked hot start polymerase
- RNAse inhibitor
- With and without UNG (Uracil-N-Glycosylase)
- With and without ROX reference dye

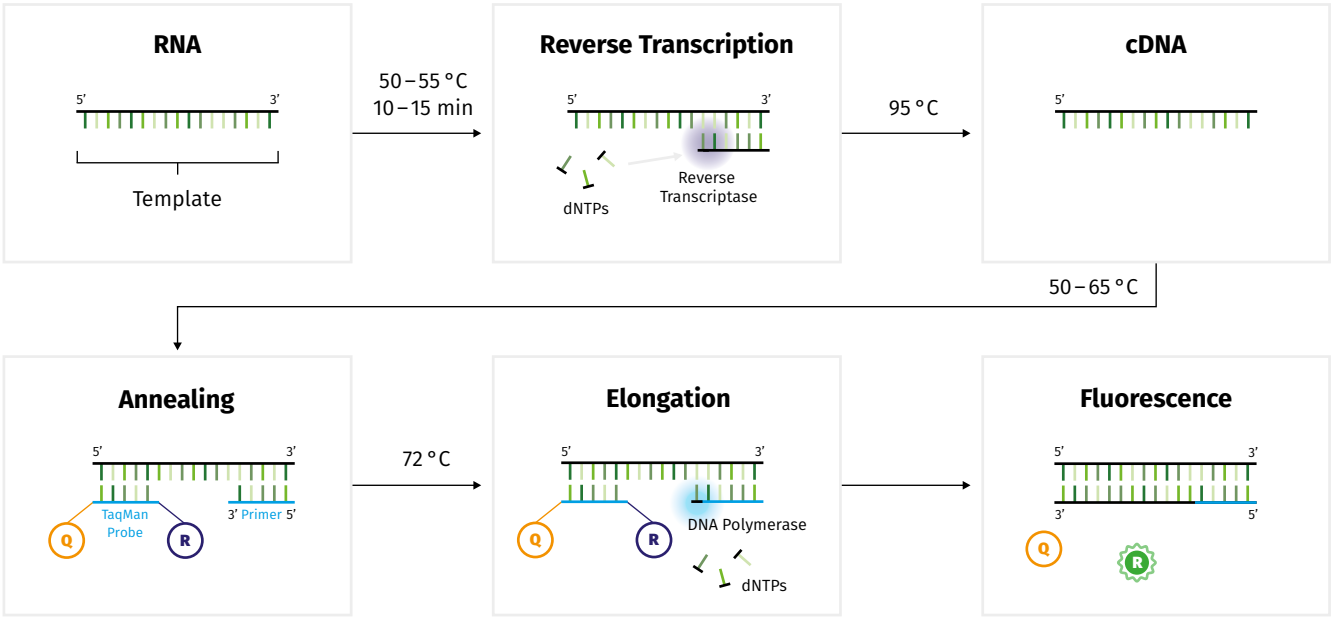
Target Detection:

- <1 pg – 20 ng poly(A) RNA
- 10 pg – 1 µg total RNA

Cat.-No.	Amount	Conc.	Reactions
SCRIPT RT-qPCR SybrMaster			
PCR-520S	2 × 1.25 ml	2 ×	250 reactions × 20 µl
PCR-520L	10 × 1.25 ml	2 ×	1,250 reactions × 20 µl
SCRIPT RT-qPCR SybrMaster highROX			
PCR-522S	2 × 1.25 ml	2 ×	250 reactions × 20 µl
PCR-522L	10 × 1.25 ml	2 ×	1,250 reactions × 20 µl
SCRIPT RT-qPCR SybrMaster UNG			
PCR-526S	2 × 1.25 ml	2 ×	250 reactions × 20 µl
PCR-526L	10 × 1.25 ml	2 ×	1,250 reactions × 20 µl

TaqMan® RT-qPCR

Probe based with high sensitivity and specificity



In a first step, the RT-qPCR mixture with reverse transcriptase, DNA polymerase and dNTPs generates cDNA from an RNA template and a corresponding gene-specific primer.

In the second step, the cDNA is amplified by PCR. DNA polymerase prolongs the DNA and its exonuclease activity degrades the dual labeled probe (TaqMan). As the reporter dye is no longer in close proximity to the quencher, the resulting increase in reporter emission intensity is detected.

SCRIPT RT-qPCR PROBESMASTER KITS

Features:

- All reagents included (just add template, primer and probe)
- Reverse transcriptase with enhanced thermal stability
- Antibody-blocked hot start polymerase
- RNAse inhibitor
- With and without UNG (Uracil-N-Glycosylase)
- With and without ROX reference dye

Target Detection:

- <1 pg – 20 ng poly(A) RNA
- 10 pg – 1 µg total RNA

Cat.-No.	Amount	Conc.	Reactions
SCRIPT RT-qPCR ProbesMaster			
PCR-512S	2 × 1.25 ml	2 ×	250 reactions × 20 µl
PCR-512L	10 × 1.25 ml	2 ×	1,250 reactions × 20 µl
SCRIPT RT-qPCR ProbesMaster highROX			
PCR-513S	2 × 1.25 ml	2 ×	250 reactions × 20 µl
PCR-513L	10 × 1.25 ml	2 ×	1.250 reactions × 20 µl
SCRIPT RT-qPCR ProbesMaster UNG			
PCR-523S	2 × 1.25 ml	2 ×	250 reactions × 20 µl
PCR-523L	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.

DIRECT AMPLIFICATION

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

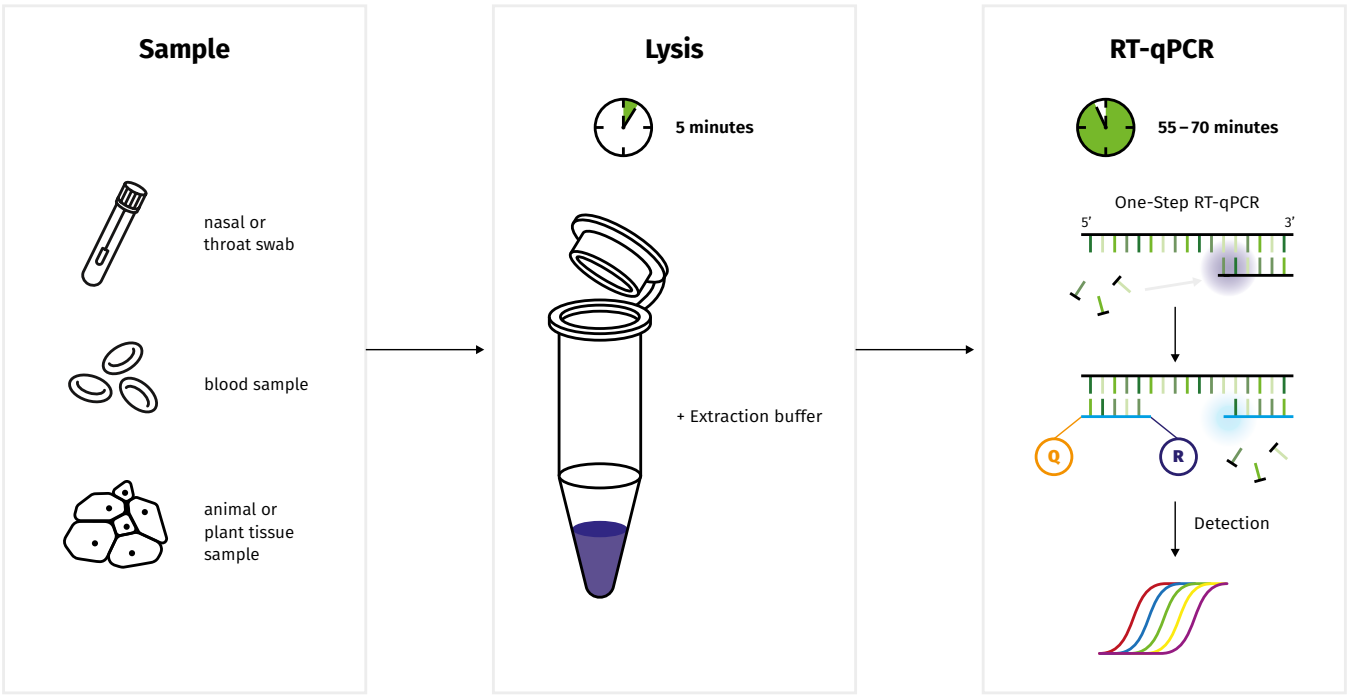
Pro

- 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

Method of Direct Amplification



Cat.-No.	Amount	Conc.	Reactions
SCRIPT Direct RT-qPCR SybrMaster			
PCR-532S	2 × 1.25 ml	2 ×	250 reactions × 20 µl
PCR-532L	10 × 1.25 ml	2 ×	1,250 reactions × 20 µl
SCRIPT Direct RT-qPCR SybrMaster highROX			
PCR-533S	2 × 1.25 ml	2 ×	250 reactions × 20 µl
PCR-533L	10 × 1.25 ml	2 ×	1,250 reactions × 20 µl
SCRIPT Direct RT-qPCR ProbesMaster			
PCR-528S	2 × 1.25 ml	2 ×	250 reactions × 20 µl
PCR-528L	10 × 1.25 ml	2 ×	1,250 reactions × 20 µl
SCRIPT Direct RT-qPCR ProbesMaster highROX			
PCR-529S	2 × 1.25 ml	2 ×	250 reactions × 20 µl
PCR-529L	10 × 1.25 ml	2 ×	1,250 reactions × 20 µl
SCRIPT Direct RT-qPCR ProbesMaster UNG			
PCR-530S	2 × 1.25 ml	2 ×	250 reactions × 20 µl
PCR-530L	10 × 1.25 ml	2 ×	1,250 reactions × 20 µl

LYOPHILISATES

Lyophilized reagents for long term storage at room temperature

- → Ready-to-use reagents pre-aliquoted with all required components
- → No cooling-chain required
- → Stable at ambient temperature
- → Reduced contamination risk

Lyophilisation Flyer

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

Cat.-No.	Amount	Conc.
SCRIPT RT-qPCR SybrMaster Lyophilisate		
PCR-169S	192 reactions × 20 µl	2 ×
PCR-169L	960 reactions × 20 µl	2 ×
SCRIPT RT-qPCR ProbesMaster Lyophilisate		
PCR-159S	192 reactions × 20 µl	2 ×
PCR-159L	960 reactions × 20 µl	2 ×

Lyophilisation Service

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

CLASSICS

Ready-to-use kits for reverse transcription and RT-PCR

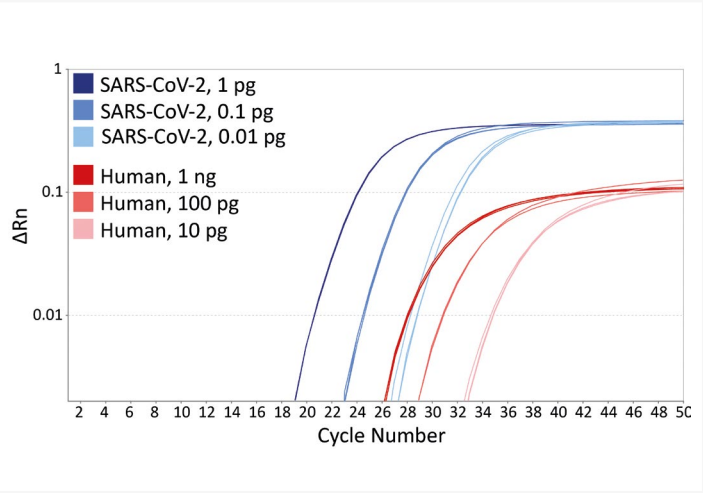
Cat.-No.	Amount	Conc.	Reactions
SCRIPT cDNA Synthesis Kit – First strand cDNA synthesis with high sensitivity and efficiency			
PCR-511S			100 reactions × 20 µl
PCR-511L			500 reactions × 20 µl
SCRIPT RT-PCR Master (2x) – One-Step RT-PCR Master Mix for highly sensitive and specific amplification			
PCR-525S	2 × 1.25 ml	2 ×	250 reactions × 20 µl
PCR-525L	10 × 1.25 ml	2 ×	1,250 reactions × 20 µl
SCRIPT High Fidelity RT-PCR Kit – One-Step RT-PCR Kit for highly precise and fast amplification			
PCR-510S			100 reactions × 50 µl
PCR-510L			500 reactions × 50 µl

SCRIPT REVERSE TRANSCRIPTASE

Increased thermal stability and sensitivity

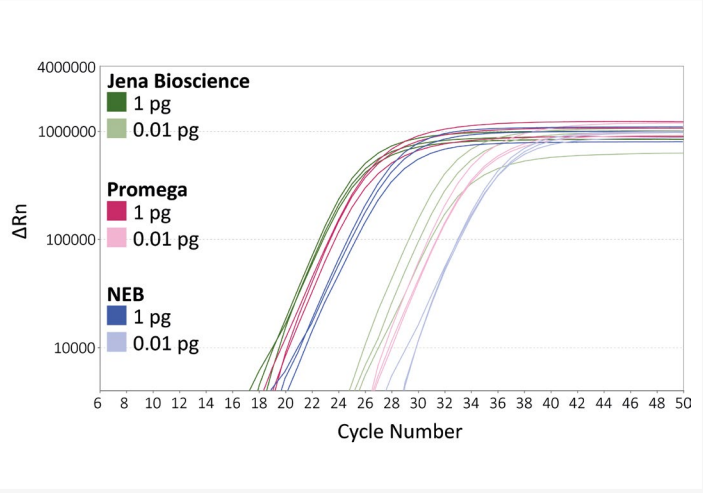
Features:

- Eliminated RNAse H activity
- Synthesis of highly structured and long cDNA fragments
- Synthesis of cDNA from 100 bp to 10 kb length



Reproducibility and Quantification

Low variability levels with various starting amounts of RNA template. Amplification plot of ß-actin transcript from human total RNA and SARS-CoV-2 RdRP transcript for detection of viral RNA. **RT-qPCR SybrMaster #PCR-522** was used for one-step RT-qPCR. Starting RNA amount was 1 pg, 0.1 pg and 0.01 pg of viral RNA and 1 ng, 100 pg and 1 pg of human RNA.



Benchmark Testing against Competitors

Amplification of a RdRP gene fragment from SARS-CoV-2 RNA. Comparison with competitors using different amounts of SARS-CoV-2 RNA as template (1 pg and 0.01 pg in triplicates). **SCRIPT RT-qPCR ProbesMaster #PCR-512** was used for one-step RT-qPCR. The Jena Bioscience master mix shows a higher sensitivity compared to other suppliers.

Cat.-No.	Amount	Conc.
SCRIPT Reverse Transcriptase		
PCR-505S	20,000 units*	200 units/µl
PCR-505L	100,000 units*	200 units/µl
PCR-505-1MU	1,000,000 units*	200 units/µl

*One unit is defined as the amount of enzyme required to catalyze the incorporation of 1 nmol of dTTP into an acid-insoluble form in 10 minutes at 37 °C.

Did you know? – RNAse H activity

RNAse H cuts RNA from RNA-DNA hybrids causing truncated cDNA. When producing long transcripts (e.g. for cloning) it is advantageous to eliminate the RNAse H activity.

COMPONENTS

Single components and additives to enhance functionality and tools for quality control

Cat.-No.	Amount	Conc.
SYBR® Green Fluorescent DNA Stain – DNA intercalation dye for Real-Time PCR analysis		
PCR-378	500 µl	100 µM
ROX Reference Dye – Reference dye for fluorescence signal normalization		
PCR-351	1 ml	25 µM
Extreme Thermolabile UNG (Uracil N-Glycosylase) – Prevention of carry-over contaminations in RT-PCR assays		
PCR-429-1KU	1 kilo unit	1 units/µl
PCR-429-10KU	10 kilo units	1 units/µl
Direct Extraction Buffer		
PCR-534S	15 ml	10 ×
PCR-534L	100 ml	10 ×
PCR-grade Water		
PCR-258S	10 ×1.2 ml	
PCR-258L	50 ml	
PCR-258-100	100 ml	
PCR-258-500	500 ml	
PCR-258-1L	1 l	

RNASE INHIBITOR

Prevent your RNA from being degraded

Applications:

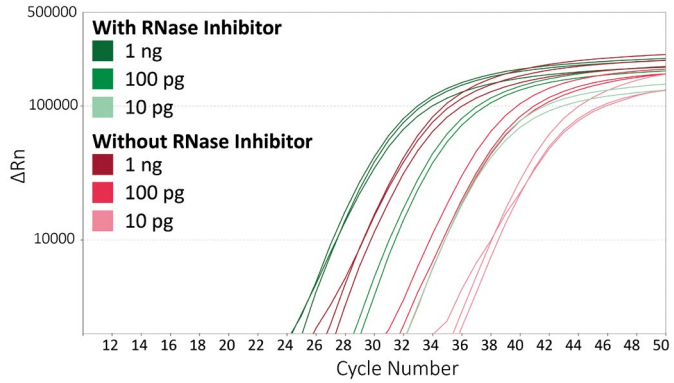
- cDNA synthesis / RT-PCR
- *In vitro* transcription/translation
- RNA purification
- RNA protection assays
- Separation and identification of specific ribonuclease activities
- Other applications where the integrity of RNA is essential

Did you know? – RNAse Inhibitor in our Master Mixes

All our RT-qPCR Master Mixes contain RNAse inhibitor

Avoiding Loss of Sensitivity due to contamination with RNase

Comparison of RT-qPCR with and without RNAse inhibitor. Amplification plot of ß-actin transcript with different amounts of total human RNA as template (1 ng, 100 pg and 10 pg in triplicates). 2 pg of RNase were added to the 20 µl RT-qPCR assay.

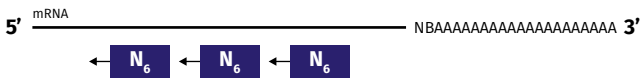


Cat.-No.	Amount	Conc.
RNase Inhibitor – recombinant		
PCR-392S	2,000 units	40 units/µl
PCR-392L	5 × 2,000 units	40 units/µl
PCR-392-100KU	2 × 50 kilo units	40 units/µl

RANDOM AND OLIGO(dT) PRIMERS

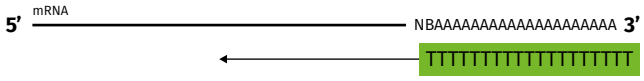
Four different types of primers can be used in the Reverse Transcriptase reaction

Random hexamer (or octamer) primer



→ bind throughout the entire length of RNA

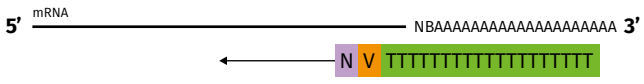
Oligo(dT) primer



→ bind randomly to the poly(A) tail of mRNA

Anchored oligo(dT) primer

N = any base
V = dGTP, dATP or dCTP



→ bind at the beginning of the poly(A) tail of mRNA

Gene specific primer

→ directing all of the RT activity to a specific sequence

Which primer should I use?

Criterion	Random Primers	(Anchored) Oligo (dT) Primers	Gene Specific
Features	Oligonucleotides with random base sequences	Stretch of 12-18 deoxythymidines	Specific to your gene sequence
One-Step RT			•
Two-Step RT	•	•	
Transcription of nonpolyadenylated RNA	•		•
Analysis of several target regions	•		
RT of partially degraded RNA	•		
Full-length RT of long RNA		•	

It is also possible to use a mixture of both random hexamers and oligo(dT) primers during cDNA synthesis.

Cat.-No.	Amount	Conc.	Sequence
Random Hexamers			
PM-301S	200 µl	100 µM | 20 nmol, 37 µg	5'- NNN NNN -3'
PM-301L	5 × 200 µl	100 µM | 100 nmol, 185 µg	
Random Octamers			
PM-302S	200 µl	100 µM | 20 nmol, 50 µg	5'- NNN NNN NN -3'
PM-302L	5 × 200 µl	100 µM | 100 nmol, 250 µg	
Oligo $(dT)_{15}$			
PM-303S	200 µl	100 µM | 20 nmol, 90 µg	5'- TTT TTT TTT TTT TTT -3'
PM-303L	5 × 200 µl	100 µM | 100 nmol, 450 µg	
Oligo $(dT)_{20}$			
PM-304S	200 µl	100 µM | 20 nmol, 120 µg	5'- TTT TTT TTT TTT TTT TTT TT -3'
PM-304L	5 × 200 µl	100 µM | 100 nmol, 602 µg	
Anchored Oligo $(dT)_{20}$			
PM-305S	200 µl	100 µM | 20 nmol, 133 µg	5'- TTT TTT TTT TTT TTT TTT TTV N -3'
PM-305L	5 × 200 µl	100 µM | 100 nmol, 664 µg	

DUAL LABELED PROBES

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

- 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
- 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 accurate measurements	
Spectral characteristics	Choose reporter dyes with minimal spectral overlap for multiplexing	Dark quencher are often preferred for multiplexing

Selecting the Reporter Dye and Quencher

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

Contact our RT-qPCR experts

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

Dr. Bürk Schäfer
Head of Molecular Biology Department

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