Instructions for Use
Life Science Kits & Assays

innuSCRIPT One Step RT-PCR SyGreen Kit

analytikjena
An Endress+Hauser Company

Content

1 Product and order number

Name	Amount	Order-no.
innuSCRIPT One Step RT-PCR SyGreen Kit	100 rxn a 20 µl	845-RT-6000100
innuSCRIPT One Step RT-PCR SyGreen Kit	200 rxn a 20 µl	845-RT-6000200

2 Storage conditions

Store innuSCRIPT One Step RT-PCR SyGreen Kit at -20 °C in a freezer with constant temperature conditions and protect from light. If stored as recommended the innuSCRIPT One Step RT-PCR SyGreen Kit is stable for at least 12 months.

3 Description

The innuSCRIPT One Step RT-PCR SyGreen Kit has been developed for fast, highly reproducible real-time PCR and has been validated on commonly used real-time PCR instruments. It contains all reagents required for One Step real-time PCR and is designed to achieve excellent results in reaction efficiency, correlation coefficient and slope.

The Mix was designed for efficient cDNA synthesis and subsequent qPCR in a single tube. It can be used to quantify any RNA template including mRNA, total RNA and viral sequences. Extremely low copy number targets can be detected specifically with high efficiency. The Mix uses a proprietary intercalating dye which does not inhibit real-time PCR, unlike other popular fluorescent dyes.

The proprietary technology prevents formation of primer dimers and non-specific products leading to improved reaction sensitivity and specificity. Only the template and primers need to be added to the reaction and the final volume should be filled up with PCR-grade water.

3.1 Quality data

Activity and stability was tested by real-time PCR. Neither human and bacterial DNA nor activity of DNase and RNase could be detected.

3.2 Instruments

Many modern real-time PCR instruments have a fast cycling mode. The improvements in sensitivity and consistency of innuSCRIPT One Step RT-PCR SyGreen Kit found in standard cycling conditions can also be used in fast and ultra fast cycling conditions.

The following real-time PCR instruments were successfully tested:

Company	*Instrument*
Analytik Jena	qTower 2.0
Biometra GmbH	TOptical
Applied Biosystems	7500, 7500 FAST, Viia7™, StepOne™, StepOne™ Plus
Bio-Rad®	iCycler®, MyiQ®, iQ™5, Opticon™, Opticon™2, Chromo4™, MiniOpticon™, CFX96™, CFX384™
Cepheid®	Smartcycler®
Eppendorf	Mastercycler® ep realplex, Mastercycler® realplex 2S
Illumina®	Eco™
Qiagen/Corbett	RotorGene™ 3000, 6000, Q
Roche Applied Science	Lightcycler®480, Lightcycler®Nano
Stratagene (Agilent)	MX 4000P®, MX 3000P®, MX 3005P®
Takara	Cycler Dice®
Techne	Quantica®

4 Delivered components

qPCR One Step Mix SyGreen 

Concentration: 2x concenctrate (with LowRox)

RTase 

Concentration: 20x (with RNase Inhibitor Mix)

5 Real-time PCR conditions for cDNA synthesis

5.1 Setup of basic real-time PCR

- Mix the following components in a thin-walled PCR tube on ice:

Component	Volume	Final concentration
PCR-grade H_2O	Variable (add to a final vol. of 20 µl)	
2x qPCR One Step Mix SyGreen	10 µl	1x
20x RTase	1 – 2 µl	(see “Note” below)
Forward primer	Variable	0.2 – 1 µM
Reverse Primer	Variable	0.2 – 1 µM
Template RNA	Variable	1 - 100 ng/µl, ≤ 1 µg)
Total volume	20 µl	

Note: We recommend to use 1 µl of RTase. 2 µl of RTase may increase primer dimers but improves Ct.

- After pipetting mix the components of the reaction mix by gently vortexing and briefly centrifugation for a few seconds to collect the mixture at the bottom of the tube.
- Reserve plate positions for positive (control RNA) and negative (water or buffer) controls.
- When preparing mixes, always calculate the volume according to the number of reactions that you need plus one extra.

Note: Reaction conditions (incubation temperatures and times, concentrations of template RNA, primers) depend on template and primers used.

5.2 Recommended time and temperature protocol

Step	Cycle	Profile	Temperature	Time
1	1	Reverse Transcription	45 - 55 °C	10 min
2	1	Initial activation	95 °C	2 - 3 min
3	40	Denaturation	95 °C	5 sec
		Annealing/ Elongation	60 - 65 °C	20 - 30 sec

Note: At the Annealing/Elongation Step we recommend not to exceed 30 sec and not to use temperatures below 60 °C. Melt Analysis: refer to instrument instructions.

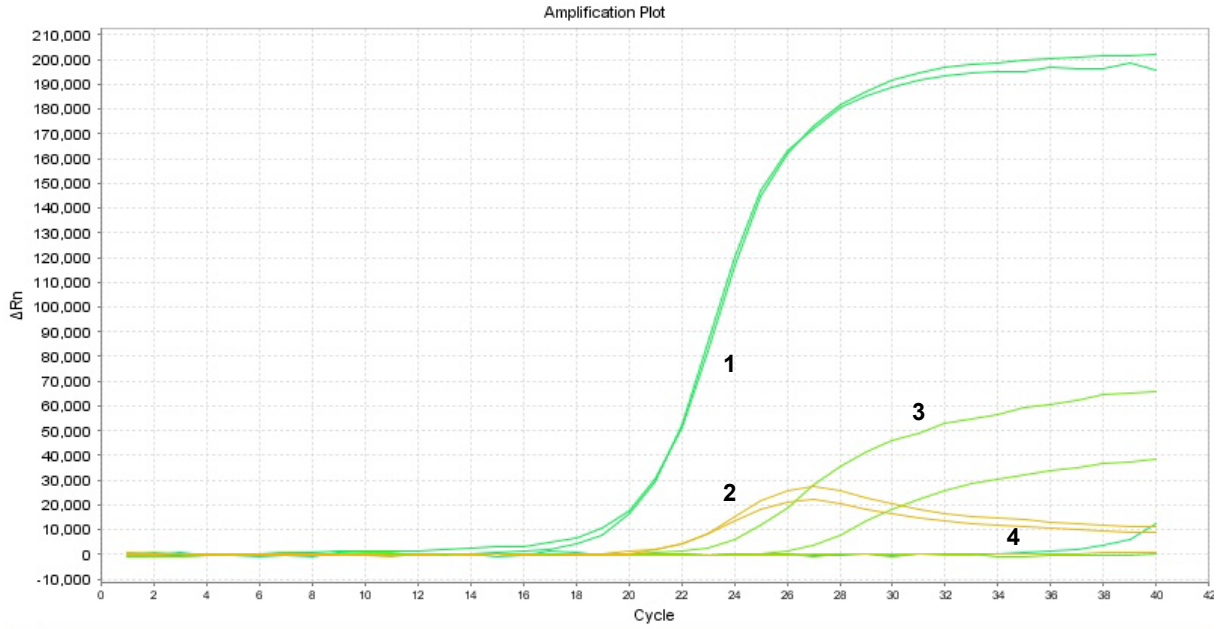
- For efficient amplification under fast cycling conditions we recommend amplicon lengths between 80 bp and 200 bp.
- The shorter the amplicon length the faster the reaction can be cycled.
- Amplicon lengths should not exceed 400 bp.
- qPCR is a very sensitive DNA amplification reaction, therefore care should be taken to eliminate the possibility of contamination with any foreign RNA templates or PCR products.
- Measure in SyGreen channel for your target sequence and in ROX channel for reference dye.

6 Related products

Product	Amount	Order Number
innuMIX qPCR MasterMix SyGreen	100 rxn a 20 µl	845-AS-1300100
	200 rxn a 20 µl	845-AS-1300200
0.2 ml thin-walled tubes	500 tubes	844-70010-0
0.5 ml thin-walled tubes	500 tubes	844-70015-0

7 Application examples

Detection of the amplified products of cDNA synthesis of viral RNA using the innuSCRIPT One Step RT-PCR SyGreen Kit and two competitors.



Nr.	Kit	Ct-value 1	Ct-value 2
1	innuSCRIPT One Step RT-PCR SyGreen Kit	18,73	19,19
2	Competitor 1	23,16	22,98
3	Competitor 2	28,15	24,58
4	negative controls	no Ct	no Ct

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This documentation describes the state at the time of publishing. It needs not necessarily agree with future versions. Subject to change!

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