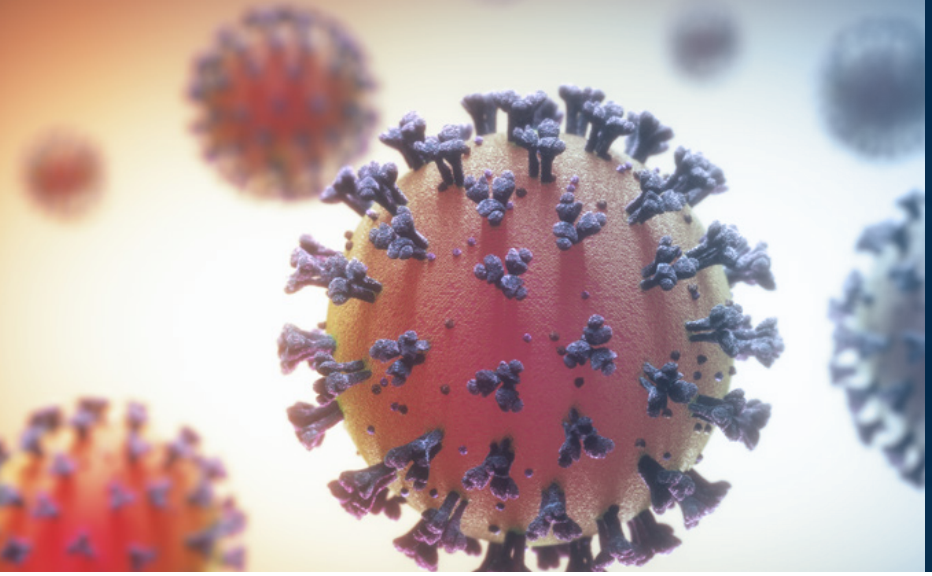

Reagents for 2019 Coronavirus (COVID-19)

The coronavirus disease (COVID-19) outbreak that began in Wuhan, China in December 2019 was declared a pandemic by the WHO on March 11, 2020. It is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and it is highly contagious, spreading via direct contact with respiratory droplets.

COVID-19 is a single-stranded RNA virus which enters a human cell via an interaction between its spike protein that protrudes from the viral outer coat and a human cell surface protein known as angiotensin converting enzyme II (ACE II). Virus genome sequencing of COVID-19 shows 88% identity to the sequence of two bat-derived severe acute respiratory syndromes (SARS)-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21 and about 50% identity to the sequence of MERS-CoV. *(https://www.sciencedirect.com/science/article/pii/S2095177920302045).* Clinical diagnosis is based on epidemiological history, clinical manifestations and nucleic acid detection.

Bioline has a comprehensive portfolio of molecular reagents to help advance your research on COVID-19. From RNA extraction solutions based on silica columns, through to 1-step or 2-step RT-qPCR formulations for fast and efficient cDNA synthesis and qPCR, we have the tools to simplify, accelerate and improve your life science research.

Sample Preparation

ISOLATE II RNA Mini Kit provides a simple, efficient column-based method for the isolation of total RNA from biological liquids.

- Fast – simple extraction of high purity total RNA in as little as 30 minutes
- High-performance – recovery of consistently high-quality RNA
- Convenient – includes all necessary components, including filters (shredders) and DNase I

Mitchell, A. B., *et al.* "A novel sampling method to detect airborne influenza and other respiratory viruses in mechanically ventilated patients: a feasibility study." *Annals of intensive care* 8.1 (2018): 45.

Karkashan, A. *et al.* "Influenza virus vectors: Stability of generated recombinant virus." *J. Current Research* 11.04 (2019): 2999-3005

cDNA Synthesis

SensiFAST cDNA kit provides a rapid and sensitive method for first-strand cDNA synthesis, which displays excellent linearity across a wide range of starting material. This gives the same relative representation in cDNA templates, regardless of gene abundance, making it excellent for use in qPCR studies.

- Efficient – high-target affinity, for improved yield of full-length cDNA
- Unbiased – optimized mix of primers for complete 5' to 3' RNA sequence representation
- Sensitive – enabling accurate detection of very low-copy targets

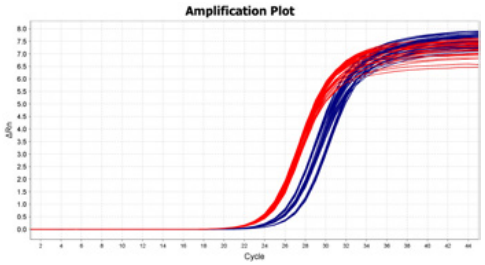

Fig 1. Unbiased representation across target genes

SensiFAST cDNA Synthesis Kit and a kit from supplier B were used in a first-strand reaction containing total RNA. Primer pairs were designed at 1 kb intervals across the same transcript and used in a qPCR reaction. The results illustrate that unlike the results from supplier B (blue). SensiFAST cDNA Synthesis Kit (red) did not show any bias across the intervening transcript.

Caly, L., *et al.* "Isolation and rapid sharing of the 2019 novel coronavirus (SAR-CoV-2) from the first diagnosis of COVID-19 in Australia." *Med J. Aust* online: 9 March 2020.

Naga, Iman S., *et al.* "Human coronavirus OC43 and other respiratory viruses from acute respiratory infections of Egyptian children." *Acta Microbiologica et Immunologica Hungarica* (2020): 1-8.

Two-Step qPCR

SensiFAST Probe Kit has been developed for fast, highly reproducible qPCR under fast thermal cycling conditions.

- Reproducible – consistent results for increased confidence in results
- Robust – reliable, accurate detection of targets from a broad range of sample types
- Sensitive – quantification of low abundance targets and scarce samples

SensiFAST Lyo-Ready No-ROX Mix is a glycerol-free mix that can be lyophilized with assay-specific primers and probes to produce ambient temperature stable qPCR master mixes that give outstanding assay reproducibility, sensitivity and robustness following rehydration.

- Glycerol-free - with a novel blend of lyo-excipients for extended room-temperature stability
- Robust - Suited for multiplex-assays and low-copy number targets
- Fast - delivers reproducible, accurate assay results in as little as 30 minutes

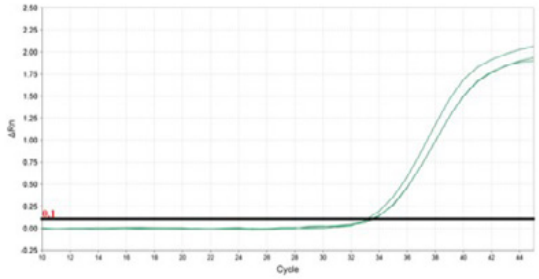

Fig 2. Increased sensitivity from lyophilization

SensiFAST Lyo-Ready No-ROX Mix was lyophilized with primers and probes to cytomegalovirus. Lyophilization maximises the amount of sample that can be added, increasing the sensitivity and as the results illustrate, it allows as few as five viral particles to be detected in a reaction.

One-Step RT-qPCR

SensiFAST Probe One-Step Kit has been optimized for fast, efficient, unbiased cDNA synthesis and subsequent highly sensitive, reproducible real-time PCR detection in a single tube. SensiFAST Probe One-Step has been optimized to deliver excellent results in both singleplex and multiplex assays.

- Sensitive – reliable quantification from even very low copy number RNA targets
- Reproducible – for increased confidence in results
- Robust – reliable detection of RNA targets from a broad range of sample types

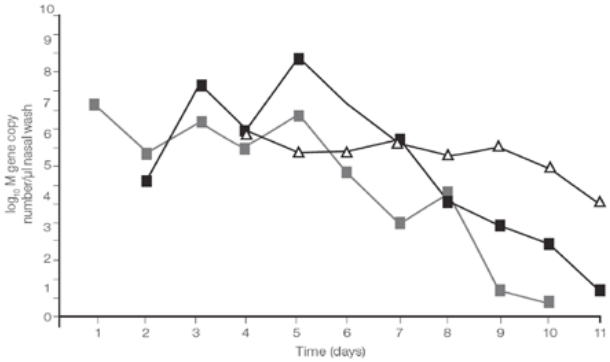

Fig 3. Copy number of viral RNA in nasal washes

WHO Collaborating Centre for Reference and Research on Influenza in Australia used RT-qPCR analysis of a mixed population of influenza viruses in ferret nasal washes to measure the viral replication and transmission kinetics of each virus population *(Butler et al. 2014).* The copy number of viral RNA in each nasal wash was determined over 11 days using SensiFAST Probe One-Step Kit. The results illustrate the sensitivity of SensiFAST Probe One-Step Kit, down to less than 1 viral particle per microliter of nasal wash.

Noh, J. Y., *et al.* "Simultaneous detection of severe acute respiratory syndrome, Middle East respiratory syndrome, and related bat coronaviruses by real-time reverse transcription PCR." *Archives of virology* 162.6 (2017): 1617-1623.

Butler J., *et al.* Estimating the Fitness Advantage Conferred by Permissive Neuraminidase Mutations in Recent Oseltamivir-Resistant A(H1N1)pdm09 Influenza Viruses. *PLOS Pathogens* 10(4): e1004065. (2014)

PRODUCT	SIZE	CAT. #
ISOLATE II RNA Mini Kit	10 Preps	BIO-52071
	50 Preps	BIO-52072
	250 Preps	BIO-52073
SensiFAST cDNA Synthesis Kit	50 Reactions	BIO-65053
	250 Reactions	BIO-65054
SensiFAST Probe No-ROX Kit	500 Reactions	BIO-86005
	2000 Reactions	BIO-86020
	5000 Reactions	BIO-86050
SensiFAST Probe Lo-ROX Kit	500 Reactions	BIO-84005
	2000 Reactions	BIO-84020
	5000 Reactions	BIO-84050

PRODUCT	SIZE	CAT. #
SensiFAST Probe Hi-ROX Kit	500 Reactions	BIO-82005
	2000 Reactions	BIO-82020
SensiFAST Probe No-ROX One-Step Kit	100 Reactions	BIO-76001
	500 Reactions	BIO-76005
SensiFAST Probe Hi-ROX One-Step Kit	100 Reactions	BIO-77001
	500 Reactions	BIO-77005
SensiFAST Probe Lo-ROX One-Step Kit	100 Reactions	BIO-78001
	500 Reactions	BIO-78005
SensiFAST Lyo-Ready No-ROX Mix	10 x 10 mL	BIO-11060

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