Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Data Sheet

Research Reagent for SARS-CoV-2 RNA

NIBSC code 19/304

(Version 2, Dated 14/01/2021)

INTENDED USE

The research reagent for SARS-CoV-2 RNA is intended to be used for the development and evaluation of nucleic acid amplification technique (NAT)-based assays for the detection of SARS-CoV-2 sequences as a positive control. The material requires extraction.

This material is for research use only, and it has only been characterized in-house.

CONTENTS

Each vial contains 0.5 mL, frozen, non-infectious synthetic SARS-CoV-2 RNA packaged within Human Immunodeficiency virus type 1 (HIV-1) particles formulated in sterile Universal Buffer comprising 10 mM Tris-HCl (pH 7.4), 0.5% human serum albumin and 1% D-(+)-Trehalose dehydrate.

CAUTION

This preparation is not for administration to humans or animals in the human food chain. The human serum albumin used in the preparation of the universal buffer has been tested and found negative for HBsAg, anti-HIV and HCV RNA. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

DESCRIPTION

The source material used to prepare the research reagent 19/304 is a lentiviral vector (LVV)-based plasmid in which the HIV genes have been substituted with Wuhan-Hu-1 isolate (GenBank MN908947.3) SARS-CoV-2 using a similar approach as described in[1]. The SARS-CoV-2 genome has been divided into four overlapping fragments and inserted within the long terminal repeats of the LVV plasmid. Single nucleotide mutations have been randomly inserted in the SARS-CoV-2 sequences to prevent protein expression. The sequences of the four constructs have been deposited to GenBank: accession numbers MT299802, MT299803, MT299804, MT299805. The four LVV-SARS-CoV-2 constructs were each individually transfected into HEK293T/17 cells together with a HIV-1 packaging plasmid p8.9, kindly donated by Prof. D. Trono [2]. Particles contained in the supernatant of the transfected cells were purified by ultracentrifugation on a 20%-sucrose cushion and resuspended in universal buffer. Genome copies for each of the four types of particles were quantified using common sequences in the HIV-U5 region upstream of the SARS-CoV-2 sequences. An equimolar mix of the four particles was formulated in the Universal Buffer at the target concentration of 10^7 copies/mL.

STORAGE

Vials should be stored at -20°C upon receipt or below. Avoid freeze/thaw cycles. No stability studies have been conducted from this material yet.

USE OF THE MATERIAL

Thaw the research reagent at ambient temperature. The research reagent should be processed according to the end user's method, including an extraction step. If a dilution series is required, we recommend diluting the material prior to extraction.

REPRESENTATIVE DATA

The reference reagent 19/304 has only been characterized in-house. The following results

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are for information only, it is the end user's responsibility to assess performance of the research reagent 19/304 in their assays.

140 µL of the research reagent 19/304 was extracted using a Qiagen vRNA kit and eluted in 60 µL AE buffer. 2.5 µL of extracted RNA was loaded per reaction using Life Technologies RNA UltraSense One-Step quantitative RT-PCR kit in a final volume of 25 µL per reaction. Samples were processed on Stratagene Mx3005p instrument and analysed using MxPro software. Results in the table below are the average of three extractions, run in duplicate.

Target	mean Ct	SD	reference
RdRp	25.27	0.14	[3]
RdRp	26.68	0.22	[4]
E	24.2	0.01	[4]

In-house calibration of 19/304 against the 1st WHO International Standard for SARS-CoV-2 RNA (NIBSC catalogue number 20/146) estimates the potency to be 6.41 Log_{10} IU/mL with 95% confidence limits of 6.30 – 6.52 Log_{10} IU/mL.

REFERENCES

[1] Mattiuzzo et al., Development of lentivirus-based reference materials for Ebolavirus nucleic acid amplification technology-based assays. PLoS One, 2015 10(11): e0142751.

[2] Zufferey et al., Multiply attenuated lentiviral vector achieves efficient gene delivery in vivo.Nat Biotechnol 15: 871–875

[3] National Reference Center for Respiratory Viruses, Institut Pasteur, Paris available at : https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6_2

[4] Corman et al., Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020;25.

CUSTOMER FEEDBACK

Customer are encouraged to provide feedback on the suitability or use of the research reagent 19/304. Please send any comments to Covid19_reagents@nibsc.org.

LIABILITY AND LOSS

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CITATION

In any publication making reference to the materials, the acknowledgment should read: "The research reagent for SARS-CoV-2 RNA (NIBSC 19/304) was obtained from the National Institute for Biological Standards and Control, UK".

MATERIAL SAFETY SHEET

Physical properties (at room temperature)			
Physical appearance	White, frozen liquid		
Fire hazard	None		
Chemical properties			
Stable	Yes	Corrosive:	No
Hygroscopic	No	Oxidising:	No
Flammable	No	Irritant:	No
Other: Contains material of human origin; This product is a genetically modified material; It is the responsibility of the end user to seek local biosafety approval for the storage and handling of the material in their workplace			
Handling:	See caution section		
Toxicological properties			
Effects of inhalation:	Not established, avoid inhalation		
Effects of ingestion:	Not established, avoid ingestion		
Effects of skin absorption:	Not established, avoid contact with skin		
Suggested First Aid			
Inhalation	Seek medical advice		
Ingestion	Seek medical advice		
Contact with eyes	Wash with copious amounts of water. Seek medical advice.		
Contact with skin	Wash thoroughly with water.		
Action on Spillage and Method of Disposal			
Spillage of ampoule or vial contents should be taken up with absorbent material wetted with a virucidal agent. Rinse area with a virucidal agent followed by water. Absorbent materials used to treat spillage should be treated as biologically hazardous waste.			