Centers for Disease Control and Prevention (CDC) Atlanta GA 30329-4027

## Research Use Only CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay Primers and Probes

## NOT FOR DIAGNOSTIC TESTING USE

Reagents manufactured from these sequences may not be used for diagnostic testing under FDA's authorization of the CDC Flu SC2 Multiplex Assay.

Only primers and probes labeled for EUA use and distributed by the <u>International Reagent</u> <u>Resource</u> may be used for diagnostic testing with the CDC Flu SC2 Multiplex Assay.

These oligonucleotide sequences are intended to be used for respiratory virus surveillance and research. The recipient agrees to use them in compliance with all applicable laws and regulations. Every effort has been made to assure the accuracy of the sequences, but CDC cannot provide any warranty regarding their accuracy. The recipient may acknowledge the source of sequences in any oral presentations or written publications concerning the research project by referring to the Genomics and Diagnostics Team, Virology Surveillance and Diagnosis Branch, Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA. CDCSARS2FluAB@cdc.gov

Should the recipient use these sequences in the design of a diagnostic device, the device developed from these sequences and the use of primers and probes synthesized from these sequences, is not authorized under CDC's Flu SC2 Multiplex Assay EUA. The device would be considered a new and separate test from the CDC Flu SC2 Multiplex Assay and must comply with applicable laws and regulations. CDC has extended an open right of reference to CDC's EUA for the CDC Flu SC2 Multiplex Assay. Any recipient seeking FDA authorization for a device based in whole or in part on the design of the CDC Flu SC2 Multiplex Assay is welcome to reference the CDC EUA (EUA 201781) in their application.

| Tube 1: CDC Flu SC2 Multiplex Assay: Forward and Reverse Primers |             |                                     |               |  |  |
|--|-------------|-------------------------------------|---------------|--|--|
| Name   | Description | Oligonucleotide Sequence (5' to 3') | Concentration |  |  |
| InfA-F   | InfA For1   | CAA GAC CAA TCY TGT CAC CTC TGA C   | 3.33 μΜ       |  |  |
|  | InfA For2   | CAA GAC CAA TYC TGT CAC CTY TGA C   | 3.33 μΜ       |  |  |
| InfA-R   | InfA Rev1   | GCA TTY TGG ACA AAV CGT CTA CG      | 5.00 μΜ       |  |  |
|  | InfA Rev2   | GCA TTT TGG ATA AAG CGT CTA CG      | 1.67 μΜ       |  |  |
| InfB-F   | InfB For    | TCC TCA AYT CAC TCT TCG AGC G       | 6.67 μΜ       |  |  |
| InfB-R   | InfB Rev    | CGG TGC TCT TGA CCA AAT TGG         | 6.67 μΜ       |  |  |
| SC2-F  | SC2 For     | CTG CAG ATT TGG ATG ATT TCT CC      | 6.67 μΜ       |  |  |
| SC2-R  | SC2 Rev     | CCT TGT GTG GTC TGC ATG AGT TTA G   | 6.67 μΜ       |  |  |
| RP-F   | RNase P For | AGA TTT GGA CCT GCG AGC G           | 6.67 μΜ       |  |  |
| RP-R   | RNase P Rev | GAG CGG CTG TCT CCA CAA GT          | 6.67 μΜ       |  |  |

| Tube 2: Flu SC2 Multiplex Assay: Probes (only 1 probe set is required) |                               |   |               |  |  |
|--|-------------------------------|---|---------------|--|--|
| Name   | Description                   | Oligonucleotide Sequence (5' to 3')                                     | Concentration |  |  |
| Option 1: IDT Probes   |                               |   |               |  |  |
| InfA-P   | InfA Probe <sup>1</sup>       | 5'-/FAM/TGC AGT CCT /ZEN/ CGC TCA CTG GGC ACG/3IABkFQ/-3'               | 1.67 μΜ       |  |  |
| InfB-P   | InfB Probe <sup>2</sup>       | 5'-/YakYel/CCA ATT CGA/ZEN/ GCA GCT GAA ACT GCG GTG/3IABkFQ/-3'         | 1.67 μΜ       |  |  |
| SC2-P  | SC2 Probe <sup>3</sup>        | 5'-/TexRd-XN/ATT GCA ACA/TAO/ ATC CAT GAG CAG TGC TGA CTC/3IAbRQSp/-3'  | 1.67 μΜ       |  |  |
| RP-P   | RNase P<br>Probe <sup>4</sup> | 5'-/CY5/TTC TGA CCT /TAO/ GAA GGC TCT GCG CG/3IAbRQSp/-3'               | 1.67 μΜ       |  |  |
| Option 2: BioSearch Probes   |                               |   |               |  |  |
| InfA-P   | InfA Probe <sup>5</sup>       | 5'-/FAM/TGC AGT CCT /Nova/ CGC TCA CTG GGC ACG/BHQ-1/-3'                | 1.67 μΜ       |  |  |
| InfB-P   | InfB Probe <sup>6</sup>       | 5'-/CIV-550/CCA ATT CGA/BHQ-1/ GCA GCT GAA ACT GCG GTG/C3/-3'           | 1.67 μΜ       |  |  |
| SC2-P  | SC2 Probe <sup>7</sup>        | 5'-/CalFluor610/ATT GCA ACA/Nova/ ATC CAT GAG CAG TGC TGA CTC/BHQ-2/-3' | 1.67 μΜ       |  |  |
| RP-P   | RNase P<br>Probe <sup>8</sup> | 5'-/Quasar670/TTC TGA CCT /Nova/ GAA GGC TCT GCG CG/BHQ-2/-3'           | 1.67 μΜ       |  |  |

<sup>&</sup>lt;sup>1</sup> Probe labeled at the 5'-end with the reporter molecule 6-carboxyfluorescein (FAM), with a ZEN™ quencher between the 9th and 10th nucleotide, and with an Iowa Black FQ quencher (IABkFQ) at the 3'-end (Integrated DNA Technologies, Coralville, IA).

InfA probe and primer sequences are identical to InfA sequences in the FDA-cleared CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (K200370).

- <sup>2</sup> Probe labeled at the 5'-end with the Yakima Yellow (YakYel) reporter, with a ZEN™ quencher between the 9th and 10th nucleotide, and with an lowa Black FQ quencher (IABkFQ) at the 3'-end (Integrated DNA Technologies, Coralville, IA). Probe and primer sequences are identical to InfB sequences in the FDA-cleared CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (K200370).
- <sup>3</sup> Probe labeled at the 5'-end with the Texas Red-XN (TexRd-XN) reporter, with a TAO quencher between the 9th and 10th nucleotide, and with an Iowa Black RQ quencher (IAbRQSp) at the 3'-end (Integrated DNA Technologies, Coralville, IA).
- <sup>4</sup> Probe labeled at the 5'-end with the CY5 reporter, with a TAO quencher between the 9th and 10th nucleotide, and with an lowa Black RQ quencher (IAbRQSp) at the 3'-end (Integrated DNA Technologies, Coralville, IA). Probe and primer sequences are identical to RP sequences in the FDA-cleared CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (K200730).
- <sup>5</sup> Probe labeled at the 5'-end with the reporter molecule 6-carboxyfluorescein (FAM), with a Nova quencher between the 9th and 10th nucleotide, and with a Black Hole Quencher®-1 (BHQ®-1) at the 3'-end (Biosearch Technologies, Novato, CA). InfA probe and primer sequences are identical to InfA sequences in the FDA-cleared CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (K200370).
- <sup>6</sup> Probe labeled at the 5'-end with the CIV-550 reporter, with a Black Hole Quencher®-1 (BHQ®-1) between the 9th and 10th nucleotide, and with a 3' Spacer C3 (Biosearch Technologies, Novato, CA). Probe and primer sequences are identical to InfB sequences in the FDA-cleared CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (K200730).
- <sup>7</sup> Probe labeled at the 5'-end with the Cal Fluor Red 610 reporter, with a Nova quencher between the 9th and 10th nucleotide, and with a Black Hole Quencher®-2 (BHQ®-2) at the 3'-end (Biosearch Technologies, Novato, CA).
- <sup>8</sup> Probe labeled at the 5'-end with the Quasar670 reporter, with a Nova quencher between the 9th and 10th nucleotide, and with a Black Hole Quencher®-2 (BHQ®-2) at the 3'-end (Biosearch Technologies, Novato, CA). Probe and primer sequences are identical to RP sequences in the FDA-cleared CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (K200730).

Note: Oligonucleotide sequences are subject to future changes as SARS-CoV-2 evolves.

Last updated: February 2, 2021