SuperScript IV reverse transcription protocols using SARS-CoV-2 research samples

Transport medium inhibitory effect

To test the compatibility of the transport medium and reverse transcription reagents, we recommend using control RNA and 5 µL of transport medium in an RT-qPCR test run before starting a real experiment.

The Invitrogen™ SuperScript™ IV CellsDirect™ cDNA Synthesis Kit and Invitrogen™ SuperScript™ IV VILO™ Master Mix have been tested with three viral transport media:

- Classical media (Capricorn Scientific) and AMIES medium (Deltalab) are compatible with the SuperScript IV reverse transcription (RT) reagents mentioned above.
- Xpert™ Viral Transport Medium (Cepheid) shows an inhibitory effect

Note: To minimize the inhibitory effect, dilute the transport medium 1:10 and/or 1:100.

Preparing heated NP swab samples in transport medium

Materials

- Nasopharyngeal (NP) swabs collected in 1.5–2 mL transport medium

Transfer 5 µL of NP swab sample to a 0.2 mL PCR tube, put the tube into the thermal cycler, and set the thermal cycler at 98°C for 5 min, then place on ice for at least 1 min. Prepare at least two replicates of the same sample.

Note: You may also heat the whole swab sample to 98°C and then transfer 5 µL of the heated sample to a PCR tube.

Protocol I (lysis + cDNA synthesis)

Materials

- Heated NP swabs
- SuperScript IV CellsDirect cDNA Synthesis Kit (**Cat. No. 11750150**)
- Water, nuclease-free

Before you begin

- Thaw all reagents from the SuperScript IV CellsDirect cDNA Synthesis Kit, gently invert or finger-tap each tube to mix, then place on ice.
- Mix the SuperScript IV CellsDirect Lysis Solution gently (do not vortex) to avoid formation of foam.
- Centrifuge all reagent tubes before opening.

1. Keep samples on ice, and add 24 µL of the lysis solution to each sample tube. To mix components, pipet the mixture up and down gently 5–8 times. Ensure the lysis solution completely covers the samples.

2. Add 8 µL of SuperScript IV RT Master Mix and 3 µL of nuclease-free water to the sample tubes on ice, for a final RT reaction volume of 40 µL, according to Table 1.

Note: For the negative control, add 8 µL of SuperScript IV No RT Control instead of SuperScript IV RT Master Mix. Use a vortex and microcentrifuge to mix and spin down prepared mixtures.

Table 1. Protocol I components.

Component	1-reaction volume
Heated NP swab sample	5 µL
SuperScript IV CellsDirect Lysis Solution	24 µL
Gently pipet up and down 5–8 times to mix	
SuperScript IV RT Master Mix or SuperScript IV No RT Control	8 µL
Water, nuclease-free	3 µL
Total	**40 µL**

3. Load the tubes into the thermal cycler, and run the following protocol:

Step	Stage	Temperature	Time
Anneal	1	25°C	10 min
Reverse transcribe	2	50°C	10 min
Inactivate enzyme	3	85°C	5 min
Hold	4	4°C	∞

4. cDNA samples can be stored at –20°C for up to one week or at –70°C long term. Alternatively, proceed directly to qPCR or ePCR.

Protocol II (cDNA synthesis without lysis)

Materials

- NP swabs collected in 1.5–2 mL transport medium, heated or unheated
- SuperScript IV VILO Master Mix (**Cat. No. 11756050**)

1. Add 4 µL of SuperScript IV VILO Master Mix (or No RT Control) and 11 µL of nuclease-free water to tubes containing NP samples on ice, for a final reverse transcription reaction volume of 20 µL, according to Table 2.

Table 2. Protocol II components.

Component	1-reaction volume
Heated/unheated NP swab sample	5 µL
SuperScript IV VILO Master Mix or SuperScript IV VILO Master Mix No RT Control	4 µL
Water, nuclease-free	11 µL
Total	**20 µL**

2. Load the tubes into the thermal cycler, and run the following protocol:

Step	Stage	Temperature	Time
Anneal	1	25°C	10 min
Reverse transcribe	2	50°C	10 min
Inactivate enzyme	3	85°C	5 min
Hold	4	4°C	∞

3. cDNA samples can be stored at –20°C for up to one week or at –70°C long term. Alternatively, proceed directly to qPCR or ePCR.

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Protocol III (one-step RT-PCR without lysis)

Materials

- NP swabs collected in 1.5–2 mL transport medium, heated or unheated
- Invitrogen™ SuperScript™ IV One-Step RT-PCR System (**Cat. No. 12594025**)
- Water, nuclease-free

1. Keep all components, reaction mixes, and samples on ice. Then combine the following components in a 0.2 mL nuclease-free, thin-walled PCR tube on ice:

Table 3. Protocol III components.

Component	1-reaction volume
2X Platinum SuperFi RT-PCR Master Mix	25.0 µL
Forward primer, 10 µM (final concentration 0.5 µM)	2.5 µL
Reverse primer, 10 µM (final concentration 0.5 µM)	2.5 µL
SuperScript IV RT Mix	0.5 µL
Water, nuclease-free	Varies
Total	**45 µL**

2. Ensure that all of the components are at the bottom of the tube. Centrifuge briefly if needed.
3. Add 5 µL of heated/unheated NP swab sample, or nuclease-free water for a no-template control (NTC), to a thin-walled PCR tube.
4. Load the tubes into the thermal cycler, and run the following cycling protocol:

Step	Temperature	Time	Cycles
Reverse transcription	50°C	10 min	1
Enzyme inactivation/initial denaturation	98°C	2 min	
Amplification	98°C	10 sec	35–40**
	55–72°C*	30 s/kb	
	72°C	5 min	
Final extension	72°C	5 min	1

* Important: Use the T_m calculator at **thermofisher.com/tmcalculator** to determine actual annealing temperature.

** Use 40 cycles for short (≤3 kb) templates only.

Note: For one-step RT-qPCR, use the Invitrogen™ SuperScript™ III One-Step RT-PCR System with Platinum™ *Taq* DNA Polymerase (**Cat. No. 12574026**), following the WHO protocol.

Materials for downstream applications

- Applied Biosystems™ TaqMan® Fast Advanced Master Mix (**Cat. No. 4444556**)
- Invitrogen™ Platinum™ SuperFi™ II Green PCR Master Mix (**Cat. No. 12369010**)
- Invitrogen™ E-Gel™ Power Snap Electrophoresis System Starter Kit, EX DC 2% (**Cat. No. G8332ST**)

Find out more at **thermofisher.com/superscript**

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