

Supplementary Method 5 Manual Preparation of RT-PCR master mix plate

Equipment / Consumables

- Rainin L-100 manual single pipette
- Rainin L-1000 manual single pipette
- Rainin 20-300µl LTS multichannel pipette
- Rainin 10-100µl or 20-200µl LTS electronic single channel pipette
- Rainin filtered tips: green box, blue large box, and grey/yellow box
- Eppendorf Tube rack
- 25ml individually wrapped reservoirs
- PCR plate (MicroAmp™ Fast Optical 96-well reaction plate with barcode, Cat. no. 4346906)
- MicroAmp™ Optical Adhesive Film (Cat. no. 4311971)
- Seal roller
- PCR chiller plate
- Vortex

Reagents

Real-time fluorescent RT-PCR kit for detecting 2019-nCoV (BGI, 50 reactions; Cat MFG030010)*			
Item (50 tests/kit)	Specification	Quantity	Description
2019-nCoV Reaction Mix	1mL /vial	1 vial	Composed of reagent for amplification and probes and primers of target gene and internal reference
2019-nCoV Enzyme Mix	80µL /vial	1 vial	Taq polymerase, Reverse transcriptase and UDG
2019-nCoV Positive control	750µL/vial	1 vial	Mix solution of pseudo-virus with target virus genes and internal reference
2019-nCoV Blank control	750µL/vial	1 vial	(Not used in protocol)

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Procedure

In RNA extraction lab:

1. Prepare sealed and barcoded empty PCR plates and store in lab. Barcodes of one PCR plate will be scanned and linked to a barcoded RNA plate when the RNA is eluted.
2. **The coupled empty PCR plate and RNA plate will be taped together and handed over if RT-PCR staff are retrieving straight away or otherwise stored at -80°C.**
3. Take both linked plates to PCR lab. Place RNA plate on ice. Take PCR plate only to RT-PCR mix aliquoting bench.

Manual Preparation of PCR master mix in 1 x 96 well MicroAmp™ plate

NOTE: Reagents are stored at -20°C. Take out all the kit contents and thaw them thoroughly at ambient temperature. Vortex and centrifuge briefly. The enzyme mix should be kept on ice at all times

4. When email indicating 'RNA plate is ready' is received, take required number of kits from the -20°C freezer (2 Kits per 96w plate of RT-PCR reactions) and remove the *2019-nCoV Enzyme mix* and Negative Control tubes from them to maintain in -20°C freezer while the remaining tubes (*2019-nCoV Reaction mix*, Positive Control) thaw at RT.
5. When *2019-nCoV Reaction mix* has thawed, retrieve *2019-nCoV Enzyme mix* tubes from -20°C, quick spin down all tubes.
6. Using an L-100 pipette with filtered tip from green tip box, add 80µl of *2019-nCoV Enzyme mix* (entire tube) to one *2019-nCoV Reaction mix* tube to make final PCR Master mix. Mix well by vortex and spin down.
7. Dispense PCR Master mix into plates as follows:
 - For half plate, leave PCR mix in tube and use an electronic single channel pipette fitted with a filter tip to draw up 100 or 200µl and repeatedly dispense 20µl into individual wells of the 96 well PCR plate.
 - For Full Plate: Using an L-1000 pipette with filtered tip from Blue tip box, pipette all the Master Mix from individual tubes into a 25ml individually wrapped reservoir. Dispense 20ul of Master mix from step into the first 7 wells of a barcoded MicroAmp™ Fast optical 96-well plate, using an LTS 20-300µl multichannel pipette.

Pipette settings: From Main menu, select Multi-Disp setting with Aliquot volume 20 µl, 7 aliquots, 1/1 Asp/Dsp Speed

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- 8 Eject any remaining PCR master mix left in tips back into reservoir.
- 9 Use 2 tips to take up PCR Master mix and dispense into wells of last row, 2 wells at a time keeping the same pipette setting.
- 10 Seal the plates with MicroAmp™ Optical Adhesive Film. Check each well is sealed.
- 11 Briefly **spin** the plate at 1000rpm, 1min, and store on ice or at 4°C, together with the thawed positive control tube.
- 12 Transfer remaining PCR master mix back into a Reaction Mix tube, label with date, and put in box in fridge under the bench
- 13 Clean up bench by spraying with 70% ethanol or distal and wiping with paper towel.