

Provisional Protocol for Saliva sample collected in Lucence SAFER kit

Reagents:

Liquefaction reagent (1M DTT in 1x PBS) :

- Stock solution 1x PBS pH 6.8
- Prepare fresh: Weigh out 1.54g of DTT power and add into a 50ml tube. Dissolve in 8ml 1 X PBS and top up to 10ml mark with 1xPBS (this is sufficient for 12 samples)
- DTT: Goldbio CAS no (27565-41-9 / 3483-12-3)

Extraction kit:

- PerkinElmer® Nucleic Acid Extraction Kit (SY609)

Real-time PCR kit:

- PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay (COVID-19-PCR-AUS-C)

Instrument:

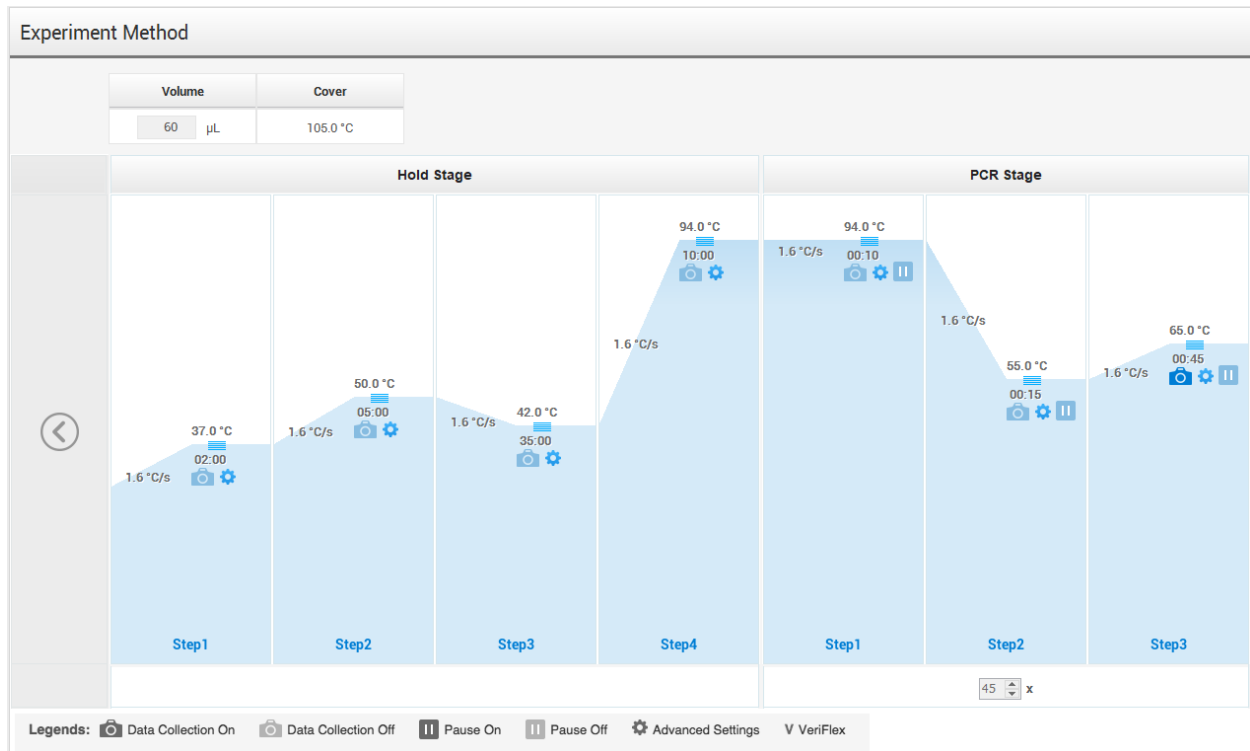
- PerkinElmer® Pre-Nat II Automated Workstation (For Extraction and PCR setup)
- Thermo Fisher Scientific QuantStudio™ 5 Real Time PCR System

Procedure

- 1) Add 750ul of liquefaction solution into each saliva sample (containing 2ml saliva and 2ml stabilization solution)
- 2) Vortex samples for 20 s and let it stand at room temperature for at least 15minutes prior to processing.
- 3) Aliquot appropriate amount of for processing. Minimal, 700ul is aliquoted to secondary tube to be processed on Pre-Nat II following manufacturer's instructions. 400ul of samples will be processed on the Pre-Nat II
- 4) Sample is eluted in 60ul of elution buffer (Tris-HCL)
- 5) Final reaction volume: 20ul of master mix and 40ul of eluted RNA.
- 6) Tubes are cap and run on QuantStudio 5 following manufacturer's instructions.

Run and analysis parameters for QuantStudio™ 5

Experiment method:



Targets assignment:

Assign Targets and Samples

Quick Setup **Advanced Setup**

Well Attributes

Sample:

Target:

Well Comments:

Plate Attributes

Passive Reference:

Quick Setup		Advanced Setup				
Targets				+ Add	✎ Action ▼	
	Name	Reporter	Quencher	Comments	Task	Quantity
<input checked="" type="checkbox"/>	N	FAM	None		U ▼	✕
<input checked="" type="checkbox"/>	IC	VIC	None		U ▼	✕
<input checked="" type="checkbox"/>	ORF1ab	ROX	None		U ▼	✕

Analysis Parameters:

Analysis Settings for SARs-CoV-2

Cr Settings | Flag Settings | Advanced Settings | Standard Curve Settings

Data Step Selection
 Select the step and stage to use for Cr analysis. Only stage/step combinations for which data suitable for Cr analysis have been collected are displayed.
 PCR Stage/Step: Stage2, Step3

Algorithm Settings
 Baseline Threshold ▼

Default Cr Settings
 Default Cr settings are used to calculate the Cr for targets without custom settings. To edit the default settings, click **Edit Default Settings**.
 Threshold: AUTO Baseline Start Cycle: AUTO Baseline End Cycle: AUTO **Edit Default Settings**

Target	Threshold	Baseline Start	Baseline End
IC	5,000	AUTO	AUTO
N	50,000	AUTO	AUTO
ORF1ab	50,000	AUTO	AUTO

Cr Settings for IC
 Cr Settings to Use: Default Settings
 Automatic Threshold
 Threshold: 5,000.0
 Automatic Baseline
 Baseline Start Cycle: 3 End Cycle: 15

Results analysis:

User would still need to check amplification curve that they follow the sigmoidal curve for proper amplification before interpretation based on the Ct value.

Control	Ct		
	N (FAM)	ORF1ab (ROX)	IC (HEX/VIC)
Negative	Undet or > 42	Undet or > 42	Ct ≤ 40
Positive	≤ 35	≤ 35	/

Undet: Undetermined;

Ct		Result interpretation
IC (VIC/HEX)	N (FAM), ORF1ab (ROX)	
≤40	Both targets Undet or >42	SARS-CoV-2 not detected
/	Both targets ≤ 42	SARS-CoV-2 detected
/	One of the targets ≤ 42	Specimen needs to be re-tested from re-extraction
>40 or Undet	Both targets Undet or >42	Invalid result, specimen needs to be re-tested from re-extraction or re-collected from patient for test.

Undet: Undetermined;

/: No requirements on the Ct value;