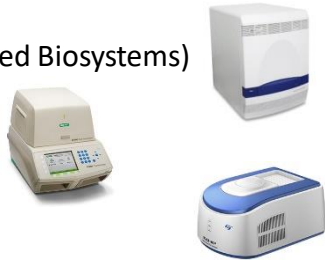


Quick Manual for PaxView® COVID-19 real-time RT-PCR Kit



| Specimen | RNA extraction | Real-time PCR machine |
|---|---|---|
| <ul style="list-style-type: none"> Oropharyngeal swab Nasopharyngeal swab Sputum Bronchoalveolar lavage fluid | <ul style="list-style-type: none"> Use CE IVD Extraction System or Kit <u>Automatic extractor</u> ex. QIASymphony® (QIAGEN) <u>Manual method</u> ex. QIAamp® Viral RNA Mini Kit (QIAGEN) | <ul style="list-style-type: none"> ABI 7500/7500Fast (Applied Biosystems) CFX96 (Bio-Rad) SLAN-96S (Hongshi)  |

Real-time PCR

- Mix PCR components

| Components | Volume |
|-----------------------------|-----------------------------|
| 4X RT-PCR (Yellow cap) | 5 μ l |
| PRIMER/PROBE MIX (Blue cap) | 5 μ l |
| Extracted RNA | 10 μ l |
| Total volume | 20 μl |



- Perform real-time PCR

| Step | Temperature | Duration | No. of cycles |
|------|-------------|----------|---------------|
| 1 | 50°C | 15 min. | 1 |
| 2 | 95°C | 10 min. | 1 |
| 3 | 95°C | 15 sec. | 40 |
| | 60°C | 45 sec. | |

- Parameter setting

| Fluorescence | Parameter | Level | | |
|--------------|-----------|---------------|----------|---------|
| | | 7500/7500Fast | CFX96-DX | SLAN96S |
| FAM | Threshold | 30,000 | 350 | 100 |
| | Baseline | Auto | Auto | Auto |
| HEX | Threshold | 5,000 | 150 | 50 |
| | Baseline | Auto | Auto | Auto |
| Cy5 | Threshold | 25,000 | 200 | 50 |
| | Baseline | Auto | Auto | Auto |

Result

| Result | Ct value | | |
|---------------------|--------------|---------|----------|
| | FAM (Orf1ab) | HEX (N) | Cy5 (IC) |
| Positive | ≤40.0 | ≤40.0 | ≤40.0 |
| Inconclusive result | ≤40.0 | No Ct. | ≤40.0 |
| | No Ct. | ≤40.0 | ≤40.0 |
| Negative | No Ct. | No Ct. | ≤40.0 |
| Invalid | No Ct. | No Ct. | No Ct. |