





SARS-CoV-2 in Extraction Buffer Inactivation Test Report

Product 1 : STANDARD™ Q COVID-19 Ag Test

Product 2 : STANDARD™ F COVID-19 Ag Test

Rev.01

Participants

Role in the study	Name	Institution	Position	Signature
Test	Jeong-Bum Kawk	BioNote, INC. R&D	M.S. Senior staff	
Test	Dong-Suk Kang	BioNote, INC. R&D	M.S. Manager	
Review	Dong-Hyuk Kim	BioNote, INC. R&D	M.S. Manager	
Confirm	Jung-Ho Kim	BioNote, INC. R&D	Ph.D Director	

Revision History

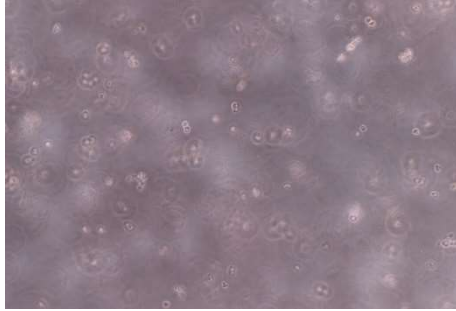
No	Contents	Date
0	First establish virus inactivation in extraction buffer study	2020. 06. 10
1	Check the minium required time for inactivation	2020. 09. 14

1. General information

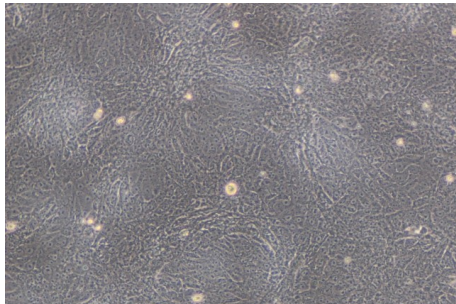
Objective	Test for virus inactivation performance evaluation of STANDARD™ Q COVID-19 Ag Test																																											
Scope	STANDARD™ Q COVID-19 Ag Test Extraction buffer (Cat No. 09COV30D) STANDARD™ F COVID-19 Ag Test Extraction buffer (Cat No. 10COV30D)																																											
Test date	2020. 09. 03 ~ 14																																											
Test site	Site : Bionote, INC. BSL-3 Laboratory Address : 22, Samseong 1-ro 4-gil, Hwaseong-si, Gyeonggi-do TEL : +82-31-211-0516 FAX : +82-31-8003-0618 E-mail : bionote@bionote.co.kr																																											
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Study method	<p>1) Extraction buffer preparation</p> <table border="1"> <thead> <tr> <th>Sort</th> <th>STANDARD™ Q COVID-19 Ag Test Extraction buffer</th> <th>STANDARD™ F COVID-19 Ag Test Extraction buffer</th> <th>Cell culture media (DMEM)</th> </tr> </thead> <tbody> <tr> <td>Manufacturer</td> <td>Biosensor</td> <td>Biosensor</td> <td>Gibco</td> </tr> <tr> <td>Lot</td> <td>STEB1020142</td> <td>FEB2120003</td> <td>2180189</td> </tr> <tr> <td>Mfg</td> <td>2020.08.28</td> <td>2020.05.26</td> <td>2020.01.22</td> </tr> <tr> <td>Exp</td> <td>2023.02.27</td> <td>2022.11.25</td> <td>2022.10.30</td> </tr> </tbody> </table> <p>2) Test procedure</p> <ol style="list-style-type: none"> ① Prepare Vero cells with ~80% confluency in 6-well plate. ② SARS-CoV-2 titer $2.5 \times 10^{4.3}$ TCID₅₀/mL with each buffer and incubation for 1 minutes interval at room temperature.. ③ After incubation, the virus-spiked buffer was inoculated into each well in which the vero cells growing at dilution 1000 -fold. ④ Culture Vero cells for 3 days at 37°C in 5% CO₂. ⑤ Check for the occurrence of CPE. ⑦ Cell supertant collected and inoculate the next passage, repeat step 5~6 <p>3) Virus preparation</p> <p>Using the SARS-CoV-2 (2019-nCoV) NCCP 43326/2020 / Korea strain cultured virus. This virus was provided by Korean CDC and cultured in Bionote BSL-3. The host cell is Vero cell and harvested after confirming 90% CPE (cytopathic effect). The titer of cultured virus was previously confirmed by TCID₅₀ and value is $1 \times 10^{6.3}$ TCID₅₀/ml.</p> <p>4) Sample preparation</p> <table border="1"> <thead> <tr> <th>.Condition</th> <th colspan="2">STANDARD™ Q COVID-19 Ag Test Extraction buffer</th> <th colspan="2">STANDARD™ F COVID-19 Ag Test Extraction buffer</th> <th colspan="2">Cell culture media</th> </tr> </thead> <tbody> <tr> <td>Virus spiking</td> <td>○</td> <td>X</td> <td>○</td> <td>X</td> <td>○</td> <td>X</td> </tr> <tr> <td>Incubation time</td> <td colspan="6">1 ~ 40 min (1 minute interval)</td> </tr> </tbody> </table>			Sort	STANDARD™ Q COVID-19 Ag Test Extraction buffer	STANDARD™ F COVID-19 Ag Test Extraction buffer	Cell culture media (DMEM)	Manufacturer	Biosensor	Biosensor	Gibco	Lot	STEB1020142	FEB2120003	2180189	Mfg	2020.08.28	2020.05.26	2020.01.22	Exp	2023.02.27	2022.11.25	2022.10.30	.Condition	STANDARD™ Q COVID-19 Ag Test Extraction buffer		STANDARD™ F COVID-19 Ag Test Extraction buffer		Cell culture media		Virus spiking	○	X	○	X	○	X	Incubation time	1 ~ 40 min (1 minute interval)					
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1) Observation by microscope

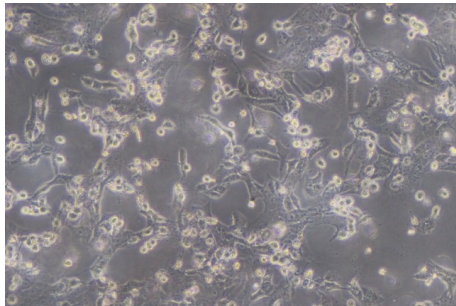
3 grade score : N/D, Non-CPE , CPE



N/D (cell lysed by ingredients from extraction)



Non CPE (Non-Cyopathic effect)



CPE (Cytopathic effect, can be detected many suspended cells)

Acceptance
Criteria

2) Criteria

Result	Interpretation
N/D	Not determine
CPE	Virus activated
Non CPE	Virus inactivated

2. Result

SARS-CoV-2 in Extraction Buffer Inactivation Test Result (live virus)					
Sample information			Microscope Observation Results * CPE : Cyto-Pathic Effect		
Extraction buffer	Virus spiking	Incubation time (minute)	Passage 1	Passage 2	Passage 3
Q Rapid COVID-19 Ag Extraction buffer	○	1	CPE 30%	CPE 90%	CPE 100%
		2	No CPE	No CPE	No CPE
		3	No CPE	No CPE	No CPE
		4	No CPE	No CPE	No CPE
		5	No CPE	No CPE	No CPE
		6	No CPE	No CPE	No CPE
		7	No CPE	No CPE	No CPE
		8	No CPE	No CPE	No CPE
		9	No CPE	No CPE	No CPE
		10	No CPE	No CPE	No CPE
		11	No CPE	No CPE	No CPE
		12	No CPE	No CPE	No CPE
		13	No CPE	No CPE	No CPE
		14	No CPE	No CPE	No CPE
		15	No CPE	No CPE	No CPE

	16	No CPE	No CPE	No CPE
	17	No CPE	No CPE	No CPE
	18	No CPE	No CPE	No CPE
	19	No CPE	No CPE	No CPE
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	21	No CPE	No CPE	No CPE
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	23	No CPE	No CPE	No CPE
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	34	No CPE	No CPE	No CPE
	35	No CPE	No CPE	No CPE
	36	No CPE	No CPE	No CPE
	37	No CPE	No CPE	No CPE
	38	No CPE	No CPE	No CPE
	39	No CPE	No CPE	No CPE
	40	No CPE	No CPE	No CPE
X	40	No CPE	No CPE	No CPE

**F FIA
COVID-19 Ag
Extraction buffer**

O

	CPE 10%	CPE 80%	CPE 100%
1			
2	No CPE	No CPE	No CPE
3	No CPE	No CPE	No CPE
4	No CPE	No CPE	No CPE
5	No CPE	No CPE	No CPE
6	No CPE	No CPE	No CPE
7	No CPE	No CPE	No CPE
8	No CPE	No CPE	No CPE
9	No CPE	No CPE	No CPE
10	No CPE	No CPE	No CPE
11	No CPE	No CPE	No CPE
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		37	No CPE	No CPE	No CPE
		38	No CPE	No CPE	No CPE
		39	No CPE	No CPE	No CPE
		40	No CPE	No CPE	No CPE
	X	40	No CPE	No CPE	No CPE
DMEM	O	40	CPE 30%	CPE 100%	CPE 100%
(Cell culture media)	X	40	No CPE	No CPE	No CPE

3. Summary

Extaction buffer	Virus spiking	Result	
STANDARD™ Q COVID-19 Extraction Buffer	○	1 minute incubation : CPE	Virus Activated
	○	2 ~ 40 minutes incubation : No CPE	Virus inactivated
	X	No CPE	Negative control
STANDARD™ F COVID-19 Extraction Buffer	○	1 minute incubation : CPE	Virus Activated
	○	2 ~ 40 minutes incubation : No CPE	Virus inactivated
	X	No CPE	Negative control
DMEM media (cell culutre media)	○	CPE	Positive control
	X	No CPE	Negative control

4. Conclusion

- 1) Positive control (DMEM media) show general virus infected CPE.
- 2) All Negative control (Without virus spiking) did not show any CPE.
- 3) In case of 1 minute incubation, virus is still activated for both Q COVID-19 Ag extraction buffer and F COVID-19 Ag extraction buffer
- 4) It's enough to inactivate the virus at least 2 minutes incubation time. The virus in extraction buffer is enough to inactivated in 2 minutes.

Hereby, I confirm that the above is true.

September , 14, 2020

Principal Investigator

Jung-Ho Kim

Signature