



2021年5月吉日

お客様各位

ロシュ・ダイアグノスティックス株式会社
ラボソリューション事業部

抽出用バッファーによるSARS-CoV-2不活性化検証結果について

拝啓 時下ますますご清栄のこととお喜び申し上げます。また、毎々格別のお引き立てを賜り厚く御礼申し上げます。

さて、弊社 SARS コロナウイルス抗原キット「SARS-CoV-2 ラピッド抗原テスト」の抽出用バッファーによるウイルス不活性化に関する検証結果を下記の通りご案内申し上げます。詳細に関しましては、添付資料をご参照ください。

ご不明な点がございましたら、弊社営業担当者までお気軽にお問い合わせください。今後ともご支援お引き立てを賜りますよう、よろしくお願い申し上げます。

敬具

記

1. 検証内容

- 韓国疾病予防管理センターから入手したSARS-CoV-2ウイルス株にて検証
- SARS-CoV-2ウイルスを抽出用バッファーにスパイクし、室温にて培養時間1～40分経過後のサンプルをそれぞれ顕微鏡で確認
- 顕微鏡下でウイルスの活性状態を3段階で評価
(N/D：判定保留、Non-CPE：ウイルス活性なし、CPE：ウイルス活性あり)

※添付資料中の製品名「STANDARD Q COVID-19 Ag Test」が、弊社「SARS-CoV-2 ラピッド抗原テスト」に該当します

2. 結果

- 陽性コントロールはウイルス活性有り
- 陰性コントロール（スパイクなし）はウイルス活性無し
- Q COVID-19 Ag抽出用バッファーにおいて、培養時間1分ではウイルス活性有り
- Q COVID-19 Ag抽出用バッファーにおいて、培養時間2分でウイルスは不活性化される

以上

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	<i>Jeong Bum Kawk</i>
Test	Dong-Suk Kang	BioNote, INC. R&D	M.S. Manager	<i>Dong-Suk Kang</i>
Review	Dong-Hyuk Kim	BioNote, INC. R&D	M.S. Manager	<i>Dong-Hyuk Kim</i>
Confirm	Jung-Ho Kim	BioNote, INC. R&D	Ph.D Director	<i>Jung ho Kim</i>

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

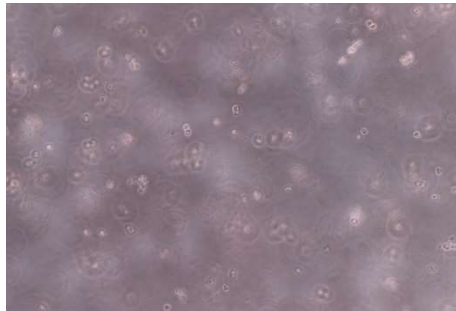
1. General information

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																																												
Participants	<table border="1"> <thead> <tr> <th>Name</th> <th>Position</th> <th>Role in the study</th> <th>E-mail</th> </tr> </thead> <tbody> <tr> <td>Jeong-Bum Kawk</td> <td>Senior staff</td> <td>Test</td> <td>jbgwak@bionote.co.kr</td> </tr> <tr> <td>Dong-Suk Kang</td> <td>Manager</td> <td>Test</td> <td>cann96@bionote.co.kr</td> </tr> <tr> <td>Dong-Hyuk Kim</td> <td>Manager</td> <td>Review</td> <td>coolvy@bionote.co.kr</td> </tr> <tr> <td>Jung-Ho Kim</td> <td>Director</td> <td>Confirm</td> <td>Jungho@bionote.co.kr</td> </tr> </tbody> </table>				Name	Position	Role in the study	E-mail	Jeong-Bum Kawk	Senior staff	Test	jbgwak@bionote.co.kr	Dong-Suk Kang	Manager	Test	cann96@bionote.co.kr	Dong-Hyuk Kim	Manager	Review	coolvy@bionote.co.kr	Jung-Ho Kim	Director	Confirm	Jungho@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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**Acceptance
Criteria**

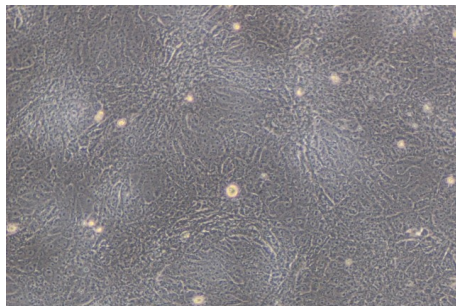
1) Observation by microscope

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



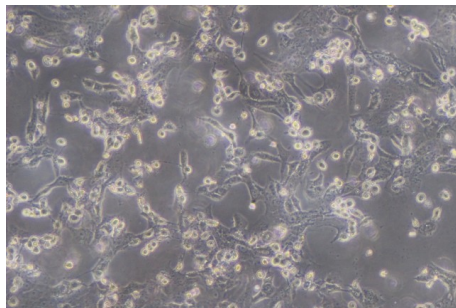
← 判定保留

N/D (cell lysed by ingredients from extraction)



← ウイルス活性なし
(ウイルスが不活性化
されたことを示す)

Non CPE (Non-Cyopathic effect)



← ウイルス活性あり

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

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
	20	No CPE	No CPE	No CPE
	21	No CPE	No CPE	No CPE
	22	No CPE	No CPE	No CPE
	23	No CPE	No CPE	No CPE
	24	No CPE	No CPE	No CPE
	25	No CPE	No CPE	No CPE
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	27	No CPE	No CPE	No CPE
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	32	No CPE	No CPE	No CPE
	33	No CPE	No CPE	No CPE
	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
12	No CPE	No CPE	No CPE
13	No CPE	No CPE	No CPE
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		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