2020-04-10

Test report

Requester: Shimanishi-Kaken Co., Ltd

By PROTECTIA, LTD
8-1 Mihogaoka, Ibaraki-shi, Osaka 567-0047
Room I213 in the Institute of Scientific
and Industrial Research, Osaka University
Nobuyuki Tanaka Chief of Testing Laboratory

Title : Evaluation of the anti-influenza virus activity of Themarox (2000-fold diluted solution)

We will report on the test results of the above sample diluents received by us on March 11, 2020.

2020-04-10

Virus inactivation test

I-1. Requester Shimanishi-Kaken Co., Ltd

I-2. Testing organization and address

Testing Institution : PROTECTIA, LTD

Test location: Room I213 in the Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki-shi, Osaka 567-0047

Exam director: Nobuyuki Tanaka

I-3. Test date April 3, 2020

I-4. Specimen used

- Themarox 2000-fold diluted solution
- Test control Phosphate buffer (PBS (-))

I-5. Test outline

The inactivation effect of the above solution on influenza virus is evaluated.

I-6. Target strains, cells and virus strains

Influenza virus H1N1 A/PR/8/34 ATCC VR-1469

Host cells: MDCK cells (canine kidney cells) ATCC CCL-34

I-7. Test method

a) 0.01 mL of Themarox was added to 0.99 mL of purified water to prepare a 100-fold diluted solution. Further, 0.15 mL of the Themarox 100-fold diluted solution was added to 2.85 mL of purified water to prepare a 2000-fold diluted solution.

b) Dispense 1.08 mL of Themarox 2000-fold diluted solution into a tube, mix 0.12 mL of influenza virus solution prepared at 5-10 x 10^5 pfu / mL with phosphate buffered saline (PBS), to prepare the test solution.

c) The test solution was allowed to stand at room temperature and reacted. At predetermined time intervals (immediately, 1 minute and 5 minutes), 0.12 mL of the solution was collected from the test solution, and mixed with 1.08 mL of Dulbecco's modified Eagle's medium containing 0.2% bovine serum(FBS-DMEM medium). Serial dilutions were added dropwise to host cells prepared in advance at a rate of 1 mL / WELL, respectively, and the cells were infected at 37 ° C. under 5% CO_2 for 1 hour.

d) After virus infection, the cell supernatant was replaced with 0.8% Oxoid agar solution, and cultured at 37 ° C. under 5% CO_2 for 2 days. After visual confirmation of plaque formation, the cells were fixed with a 5% glutaraldehyde solution, stained with methylene blue, and the virus infection titer was measured based on the measurement data of the number of plaques formed.

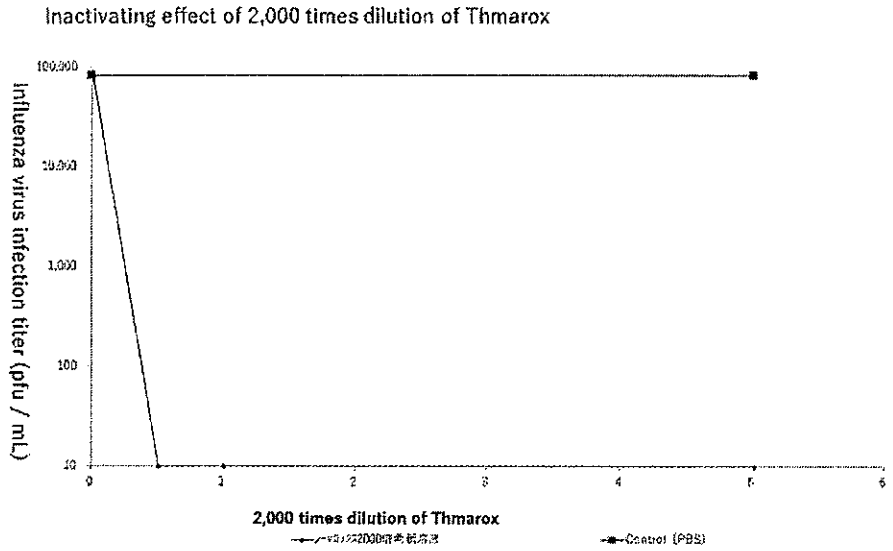
e) This test was performed twice, and based on the average value of the two tests, the inactivation rate of a 2000-fold diluted Themarox solution was evaluated.

[List of test contents]

Virus used	Influenza virus A/PR/8/34 (5-10 x 10^5 pfu / mL at the time of action)
Test time	Immediately after processing time, 1 minute, 5 minutes

2020-04-10

I-8. Test results [Influenza virus inactivating activity]



Test control	processing time			
(PBS)	Before processing	Immediately after	1 min.	5 min
Run1	77000	-	-	80500
Run2	91000	-	-	89000
Avrage	84000	-	-	84750
Decrease rate(%)	-	-	-	-0.893%

Test control	processing time			
2,000 times dilution of Thmarox	Before processing	Immediately after	1 min.	5 min
Run1	77000	<10	<10	<10
Run2	91000	<10	<10	<10
Avrage	84000	<10	<10	<10
Decrease rate(%)	-	>99.988%	>99.988%	>99.988%

I-9. Consideration and conclusion

The inactivation effect of Themarox 2000-fold diluted solution on influenza virus was evaluated. The H1N1 serotype A/PR/8/34 strain was used as the influenza virus. Immediately after the action, the Themarox 2000-fold diluted solution was confirmed to have a virus inactivating effect of 99.988% or more.

End