

Test Report

Report no : VI-R2021232S-1B
Date : 2021-07-23

Sample Number : VI-2021232S-1
Client Name : TIMELESS ETERNAL PTE LTD
Client Address : 235 Jalan Besar, #04-01, Singapore 208909
Sample Receiving Date : 2021.06.29
Testing Period : 2021.06.29 – 07.23
Sample Name : Timeless Forever NMN PURE9000
Sample Condition : The sample was received and stored at ambient temperature

Test Requested

Tranquilization Efficacy Test:
Zebrafish Embryos Motor Activity Reduction Rate



General Comments:

1. Test method was designed referring to OECD TG 236.
2. Scientific basis : Frontiers in Neural Circuits. 2013, 7: 109; Anesthesiology. 2018, 129:459-476; Biomedicines. 2019, 7, 23; Nature Communications. 2019, 10: 4078; Nature. 2019, 571(7764): 198–204.
3. Sample was pretreated according to our in-house method VI_SOP_002 as functional food product before test.

Test Results: Please refer to next page

Approved signatory:




Dr. Xueping Chen

Chief Technology Officer, UK & EU Registered Toxicologist, Chartered Biologist

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Test results:

Category: Functional food

Sample Description		Timeless Forever NMN PURE9000
Sample ID		VI-2021232S-1
Test	Concentration (g/L)	Result
Motor activity reduction rate in zebrafish embryos ^(1, 2, 3)	0.01	23% ($p = 0.000015$)
Tranquilization Efficacy ⁽⁴⁾	Significant	

Remarks:

1. Brief test description: zebrafish embryos in 5 dpf are exposed to sample solution (sample treatment) and dilution water (blank control) in 96-well plate where an embryo per well for 2 hours. The 96-well plate is placed in DanioVision, Noldus Information Technology where the zebrafish embryos are first allowed to adapt to the white light environment for 25 minutes, then the motor activity of zebrafish embryos under the alternate dark light and white light is detected and recorded every 5 minutes.
2. Motor activity reduction rate = [(the average motor activity of normal zebrafish embryos - the average motor activity of zebrafish embryos treated with testing sample) × 100 / the average motor activity of normal zebrafish embryos] %.
3. p value is obtained from the T-test between the motor activity of zebrafish embryos treated with testing sample and the motor activity of normal zebrafish embryos. Generally, $p < 0.05$ has statistically significant difference.
4. Based on the motor activity reduction rate in zebrafish embryos and the statistical difference (p value) of the reduction rate, tranquilization efficacy of a testing sample is determined as Significant (reduction rate is positive value and $p < 0.05$) or Insignificant (reduction rate is negative value or $p \geq 0.05$).
5. Refer to Appendix 1 for representative photos of tranquilization efficacy test results.
6. The test results relate only to the submitted test item with the sample number and receiving date specified on the previous page.

—End of test result—

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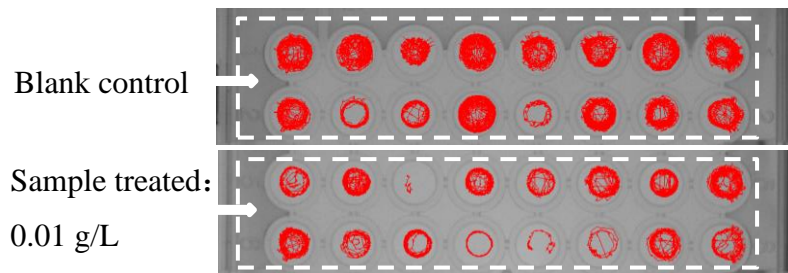
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Appendix 1.

Representative photos of Tranquilization Efficacy Test results



*Red colour represents the trajectory of zebrafish embryos.
Generally, redder colour, more active zebrafish embryos while less reddish represents more significant tranquilization efficacy.

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