

**(Direct translation from the Japanese document)**

Test Report No. 12036312001-03

Dated: 2012/08/06

Specimen

Alkaline electrolyzed water PH12. 7

Test Period

2012/06/28~2012/08/06

Test Facilities

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Test Principal

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1) Purpose of the test

In accordance with OECD Guidelines for the Testing of Chemicals 405 (2002), eye irritation  
in rabbits was investigated.

2) Samples

Alkaline electrolyzed water PH12. 7

Properties: Colorless transparent liquid

### 3) Test Animals

Japanese white male rabbits were purchased from Kitayama Labes Co., Ltd., after preliminary rearing for more than one week to confirm that there were no abnormalities in general conditions, three rabbits were used in the test. The test animals were individually housed in FRP cages and kept in a breeding room with a room temperature of 22 °C +/- 2°C with 12 hours/day of daylight. Pellet for rabbits and guinea pigs [LRC4, Oriental Yeast Industry Co., Ltd.] was given in limited quantities, and tap water was freely ingested.

### 4) Test method

The anterior segment of both eyes of each test animal was examined on the day of the start of the test to confirm that there were no abnormalities. After measuring body weight, 0.1 mL of the test substance was instilled into the conjunctival sac of one eye of each test animal, and the upper and lower eyelids were gently held together for about 1 second. The other eye was used as an untreated control. At 1, 24, 48, and 72 hours and 7 days after instillation, the cornea, iris, conjunctiva, etc. were observed using a slit lamp (X 10) [Ohira Corporation], and the degree of eye irritation was scored according to the Draize method criteria shown in Table 1. In addition, at each observation time except 1 hour after instillation, sodium fluorescein was used to observe the presence and extent of corneal epithelial damage in detail. The total score for each test animal was calculated from the obtained scores using the formula shown in Table 2, and the average total score for 3 animals was calculated for each observation time. The maximum value of the average total score during the observation period was used to evaluate the eye irritation potential of the test substance based on the criteria shown in Table 3.

### 5) Test Results (Table 4~8)

In the test eyes, redness of the eyelids and bulbar conjunctiva (both score 1) and lacrimal secretions (score 1-3) were observed in all cases from 1 hour after instillation, but disappeared by day 7. In addition, there were some cases where hyperemia of the nictitating membrane was observed. No irritation was observed in the control eyes throughout the observation period. In addition, when the test eyes and control eyes were examined with fluorescein sodium, no staining was observed at any of the observation times. The maximum value of the average total score during the observation period was 4.7 (1 hour after instillation) in the test eyes and 0 in the control eyes. The highest average total

score during the observation period was 4. It was 7 (1 hour after instillation) and 0 in the control eye.

## 6) Conclusion

An eye irritation test was conducted on rabbits using the test substance, in accordance with the OECD Guidelines for the Testing of Chemicals 405 (2002).

As a result of instilling 0.1 mL of the test substance into one eye of three rabbits, redness of the eyelids and conjunctiva of the eyeball, as well as secretion, were observed in all cases from 1 hour after instillation, but these disappeared by 7 days.

The highest value of the average total score during the observation period, calculated according to the Draize method, was 4.7 (1 hour after instillation).

Based on these results, the test substance was evaluated as being within the category of "non-irritant" in the eye irritation test using rabbits.

## 7) Bibliography

"Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics" (1959)

The Association of Food and Drug Officials of the United States.