

## Neodye DNA Green (35,000 ×)

### TOXICITY REPORT

#Cat: NB-60-0009

Size: 1ml

#### Description

This report presents a comprehensive evaluation of the toxicological properties of Neodye DNA Green (35,000 ×). The assessment encompasses a series of tests conducted to investigate the acute oral toxicity, genotoxicity, and mutagenicity of Neodye DNA Green (35,000 ×). The tests performed include the Acute Oral Toxicity Test, Mouse Marrow Chromophilous Erythrocyte Micronucleus Test, Ames Test, and *in Vitro* Mammalian Cell Chromosome Aberration Test. These tests aim to provide valuable insights into the safety profile of Neodye DNA Green (35,000×), ensuring its compliance with regulatory standards and its suitability for intended use. The findings of these tests contribute to a comprehensive understanding of the product's safety profile, facilitating informed decision-making regarding its usage in diverse applications.

#### Acute Oral Toxicity Test

#### Material and Method

##### Sample

Original liquid of Neodye DNA Green (35,000 ×), 300 mL, Brown-red

##### Animals

The animals used in the test were healthy Kunming mice, which were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Certificate No. was SCXK (Jing) 2009-0017), SPF level, body weight from 18 g to 22 g). Before testing, all animals were fasting, but water was *ad libitum*. All animals were housed in a clean animal room (CL). The room temperature was from 18°C to 22°C, and the relative atmospheric humidity ranged from 45 to 65%. The Certificate No. of the animal clean room was SCXK (Jing) 2010-0029.

##### Dose Design

According to Horn's Method, the dosages for the test groups were designed to be 1000, 2150, 4640 and 10000 mg/kg. The mice were divided into 4 groups (5 animal/sex/group) at random. Animals were administered with test item by oral gavage, the amount of exposure was calculated according to 0.2 mL/ 10 g.

##### Outcome Measures

After exposure, generally, poisoning symptoms and death were observed, the observed period was two weeks. If no mouse died in two weeks, the LD<sub>50</sub> was considered above 10000 mg/kg.

## Test result

**Table 1: Results of Acute Oral Toxicity Test with Neodye DNA Green (35,000 ×).**

Sex	Dose (mg/kg)	Number of Animals	Dead Number of Animals	Dead Rate (%)
Female	1000	5	0	0
	2150	5	0	0
	4640	5	0	0
	10000	5	0	0
Male	1000	5	0	0
	2150	5	0	0
	4640	5	0	0
	10000	5	0	0

### Conclusion

After exposure, poisoning symptom was not observed, meaning that LD<sub>50</sub> is all 10000 mg/kg. Neodye DNA Green (35,000 ×) showed to be non- toxic.

### Mouse marrow chromophilous erythrocyte micronucleus test

This test was performed to assess the effect of Neodye DNA Green (35,000 ×) on the incidence of micronuclei of bone marrow polychromatic erythrocytes of the mouse.

### Material and Method

#### Sample

Original liquid of Neodye DNA Green (35,000 ×), 300 mL, Brown-red

#### Animals

The animals used in the test were healthy Kunming mice, which were purchased from the National institute for the control of pharmaceutical and biological products The Certificate No. was SCXK (Jing) 2009-0017, cleaning level, body weight were from 25 g to 30 g.

#### Method

Mice in test groups were administrated orally with the test item twice in 30 hours. The dosages of test groups were 1000, 2000 and 5000 mg/kg, respectively. The negative control was administrated with distilled water and the positive control group was administrated with Mitomycin C (1.5 mg/kg). In each group, five males and five females mice were used. After 6 hours of the second treatment, the mice were sacrificed. The marrow of sternum was taken and the marrow suspension was made into slides. The slides were stained with Giemsa's and examined under the microscope. 1000 polychromatic erythrocytes (PCE) were observed for each animal. The number of cells with micronucleus was counted.

#### Test criteria

Hygienic Standard for Cosmetics (2007), Technical Specification for Identification of Toxic Chemicals (2005).

## Test result

**Table 2: Results of Mouse marrow chromophilous erythrocyte micronucleus test with Neodye DNA Green (35,000 ×).**

Sex	Group	Dose (mg/kg)	Number of Animals	PCEs	PCEs with micronuclei	The incidences of micronuclei(%0 )	p
Female	Neodye DNA Green (35,000 ×)	1000	5	5000	4	0.80±0.45	>0.05
		2000	5	5000	4	0.80±0.45	>0.05
		5000	5	5000	3	0.60±0.55	>0.05
	Distilled water	-	5	5000	4	0.80±0.45	-
	MitomycinC	1.5	5	5000	135	27.0±1.22	<0.01
Male	Neodye DNA Green (35,000 ×)	1000	5	5000	4	0.80±0.45	>0.05
		2000	5	5000	4	0.80±0.45	>0.05
		5000	5	5000	3	0.60±0.55	>0.05
	Distilled water	-	5	5000	3	0.60±0.55	-
	Mitomycin C	1.5	5	5000	123	24.6±3.05	<0.01

## Conclusion

The results showed that there was no significant difference ( $P>0.05$ ) in the incidence of micronuclei between the test groups and negative control while there was significant difference ( $P<0.01$ ) between Mitomycin C and negative control. That means the result of Mouse marrow chromophilous erythrocyte micronucleus test with Neodye DNA Green (35,000 ×) is negative.

## Ames Test

### Material and Method

#### Sample

Original liquid of Neodye DNA Green (35,000 ×), 100 mL, Brown-red

#### Strains

*Salmonella typhimurium* strains TA97, TA9s, TA100 and TA102 were identified by our laboratory, the strains were fulfilled the experimental conditions set up by Ames.

#### Test item

Neodye DNA Green (35,000 ×) 1M is brown-red liquid and can be dissolved in water. The test item was dissolved in sterile distilled water, and the dosages were 0.5, 1.0, 2.5, and 5 mg/mL, respectively.

#### Chemical reagents

1,8-hydroxyanthraquinone; 2-AF; 9-fluorenone; S9 (The S9 mix preparation was performed according to Ames et al and stored in -80°C), NaN<sub>3</sub> and Mitomycin C.

#### Grouping

The dosages were 0.5, 1.0, 2.5 and 5.0 mg/plate, respectively. Three parallel plates were set for different dosages. The control groups included blank control plates, solvent control plates (sterile distilled water) and positive control plates. In the absence of S9 mix, the positive control reference substance for strains TA97 and TA98 was 9-fluorenone, for TA100 was NaN<sub>3</sub>, and for TA102 was Mitomycin C. In the presence of S9 mix, the positive control reference substance for strains TA97, TA9s and TA100 was 2-AF, and for TA102 was 1,8-hydroxyanthraquinone.

#### Method

0.1 mL test solution, 0.1 mL bacterial suspension and 0.5 mL exogenous metabolic system S9 mix/ without S9 mix were mixed uniformly in the test tubes with 1.5 mL overlay agar (liquid, 45°C). The mixture was uniformly poured on the prepared underlay agar plates. After solidification, the plates were incubated for 48 h at 37°C in the constant temperature incubator and then the number of revertant colonies per plate was counted. If the number was more than twice the spontaneous revertant colonies counts and showed a dose-response relationship, the positive result could be concluded.

## Test result

**Table 3: Results of Neodye DNA Green (35,000 ×) DNA Ames test (x- ±SD).**

Dose level	TA91		TA98		TA100		TA102	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Blank control (mg/plate)	135±12	156±5	30±1	32±1	136±21	155±11	247±10	275±14
Water	132±16	155±2	30±2	32±1	146±10	158±19	248±7	275±15
0.5	142±9	141±14	31±1	32±2	137±17	154±13	261±9	266±10
1	130±5	151±13	30±1	31±2	140±14	168±10	251±8	258±6
2.5	138±13	151±7	30±1	31±1	142±12	167±7	259±13	254±5
5	136±10	159±2	31±2	32±1	152±10	172±28	254±15	282±6
Positive control (µg/plate)								
NaN <sub>3</sub> (2.5)	-	-	-	-	1283±26	-	-	-
2-AF (10.0)	-	1519±161	-	2306±234	-	1338±166	-	-
9-Fluorenone (0.2)	1475±105	-	2244±36	-	-	-	-	-
Mitomycin C (4.0)	-	-	-	-	-	-	1464±156	-
1,8-hydroxyanthraquinone (50.0)	-	-	-	-	-	-	-	1562±285

## Conclusion

Four strains including *Salmonella typhimurium* strains TA97, TA9s, TA100 and TA102 were tested by the Test Substance, no matter directly response detection or post-metabolic activation detection. No mutagenicity was observed. The result of Ames test with Neodye DNA Green (35,000 ×) is negative

## **In vitro Mammalian Cell Chromosome Aberration Test**

### **Material and Method**

#### **Sample**

Original liquid of Neodye DNA Green, 300 mL, Brown-red

#### **Cell Strains**

Chinese hamster ovary line

#### **Metabolic Activation System**

S9 mix, which is rat liver homogenate induced by both phenobarbital sodium and naphthaflavone and add some appropriate cofactors.

#### **Test Compound**

5.0 mg/mL of MEM stock solution, separately diluted to different concentration by serum-free culture solution (3h, presence and absence of S9 mix) and MEM medium supplemented with 10% fetal bovine serum (24 hours, absence of S9 mix).

#### **Method**

##### **Culture solution**

MEM medium supplemented with 10% fetal bovine serum and 100 IU/mL of penicillin and streptomycin.

##### **Maximum Final Concentration Determination**

It is shown that the metabolic activation way is +S9 and -S9, and the action time are 3 hours through two-time preliminary experiments. We also confirmed the highest final concentration of the -S9 metabolic activation way responding for 24 hours at the two different conditions. Both of the two preliminary experiments contain test group and blank control group. After high-density inoculation 96 well plate with CHO cell, 37°C, 5% CO<sub>2</sub> for 24 h, discard the medium in the plate, add test compound with different concentration and serum-free culture solution, add S9 mix into metabolic activation set, only add culture solution into blank control set, and incubate for 3 h in the constant temperature incubator. Discard the culture solution, wash cells 3 times with D-hanks, add culture solution supplemented with 10% fetal bovine serum, and continue to incubate for 24 h. Measuring cell activity with resazurin, and determine the final concentration according to cell inhibiting rate.

### Chromosome aberration test

Two kinds of test condition, one kind of it is metabolic activation is +S9 and -S9, action time is 3 h, another one is -S9, action time 24 h. In the first test condition: test compound group, negative controls and positive control group were established. According to the result of preliminary test, the final concentration of the test compound is 1250.0, 2500.0 and 5000.0 µg/mL (-S9), and 1250.0, 2500.0 and 5000.0 µg/mL (+S9). Inoculation plate with CHO cell, and inoculation density is  $1.2 \times 10^6$ /plate, 37°C, 5% CO<sub>2</sub> for 24 h, discard the medium in the plate, add test compound with different concentration and serum-free culture solution, add S9 mix into metabolic activation set, and incubate for 3 h in the constant temperature incubator. In the second test condition: inoculation density of CHO cell is  $1.0 \times 10^6$ /plate, 37°C, 5% CO<sub>2</sub> for 24 h, discard the medium in the plate, add test compound with different concentration and medium supplemented with 10% fetal bovine serum, and incubate for 24 h in the constant temperature incubator.

### Cell Harvesting

4 h before harvesting, add colchicines to 1.0 µg/mL. Hypoton, fixation, tableting and dye with Giemsa. Select 200 normal metaphase cell in test compound set and blank control set and select 100 normal metaphase cells in positive control set, then analysis the chromosome aberration. Record the chromosome aberration type and number and figure out the chromosome aberration rate.

### Statistical Analysis

Making comparison between chromosome aberration rate of each test compound and chromosome aberration rate of negative control set by x2 analysis.

### Test result

**Table 4: The results of in Vitro Mammalian Cell Chromosome Aberration Test (3 h, -S9).**

Group	Final Concentration (µg/mL)	Total Cell Number	Aberrant Cell Number	Aberration Rate (%)
Negative control	-	200	3	1.5
Test compound	1250.0	200	2	1.0
	2500.0	200	4	2.0
	5000.0	200	1	0.5
Mitomycin C	1.0	100	11	11.0*

\*  $P < 0.01$

**Table 5: The results of *in vitro* Mammalian Cell Chromosome Aberration Test (3h, +S9).**

Group	Final Concentration (µg/mL)	Total Cell Number	Aberrant Cell Number	Aberration Rate (%)
Negative control	-	200	1	0.5
Test compound	1250.0	200	0	0
	2500.0	200	0	0
	5000.0	200	0	0
Cyclophosphamide	15.0	100	13	13.0*

\*  $P < 0.01$

**Table 6: The result of *in vitro* Mammalian Cell Chromosome Aberration Test (24h, -S9).**

### Conclusion

Under the experimental conditions, *in vitro* mammalian cell chromosome aberration detection system, compared to negative control group, no matter add metabolic activation system or not, the test compound did not lead to higher chromosome aberration rate, so the result of *in vitro* mammalian cell chromosome aberration test with Neodye DNA Green (35,000 ×) is negative.