Avena Lab
Farmadria d.o.o. Since 1991.

Import and distribution for Serbia: Farmadria DOO
info@avenalab.com
+381 (0) 69 / 55 65 029
www.avenalab.com

EFFICACY TEST

Product Name	Vitamin C (Sodium ascorbyl phosphate)
Product Code	AL00064
INCI Name	Sodium ascorbyl phosphate
CAS Number	66170-10-3

Cytotoxicity test

1. Testing objectives

To evaluate the sample cytotoxicity of the sample and predict its oral toxicity.

2. Testing materials

2.1. Experimental materials

2.1.1. NIH/3T3 cell line.

2.1.2. DMEM medium, PBS, FBS, 0.25% Tyrisin (EDTA), MTT,DMSO

2.2. Sample information

Sample name	Batch number	Appearance
SAP	20200212	White powder

3. Testing methods

MTT method

4. Testing procedures

The logarithmic phase NIH / 3T3 cells were collected, and 50 μL cell suspension was added into each well in a 96 well plate. The number of cells in each well was 5000 (the marginal holes were filled with sterile PBS) and incubate the cells overnight in the incubator. At the end of culture, 50μL samples of different concentrations were added into each well, generally 5-8 concentrations and 6 multiple wells were set up. At the same time, the solvent control group and solvent blank group (only containing the culture medium with solvent, without cells) were set up. When the sample was dissolved in the conventional medium, the solvent control was the conventional medium. If the sample is dissolved in DMSO or ethanol, the solvent control is the conventional medium containing the same amount of solvent (0.5v/v). After 48 hours of incubation, 20 μL MTT solution (5mg/mL) was added into each well for 3 hours. If the sample can react with MTT, the culture medium must be discarded first, washed with PBS for 2-3 times carefully, and then add the culture medium containing MTT. After the culture, the culture medium was carefully removed, 150 μL DMSO was added into each well, and the crystal was fully dissolved by shaking at low speed on the shaking table for 10 min. The absorbance value of each well was measured at 490nm by microplate reader.

Avena Lab
Farmadria d.o.o. Since 1991.

Import and distribution for Serbia: Farmadria DOO
info@avenalab.com
+381 (0) 69 / 55 65 029
www.avenalab.com

EFFICACY TEST

Results assessment

Cell survival rate(%) = [OD (sample) - OD(blank)] / [OD (solvent control) - OD(blank)] × 100 %.

According to the relationship between concentrations and survival rates, the IC50 can be calculated by chart method or logarithmic probability unit method, and Hill function is recommended. If the relative cell activity at a certain concentration is more than or equal to 70% compared with the control group, the test substance has no cytotoxicity at this concentration.

Prediction formula acute oral toxicity :log LD50 (mg / kg) = 0.372 log IC50 (μg / mL) + 2.024

USP toxicological classification standard

Cell viability (%)	Cytotoxicological classification
≥100	0
≥80	1
≥50	2
≥30	3
≥0	4

GHS acute toxicity classification standard

Classification	Rat oral(mg/kg)
1	LD50≤5
2	5＜LD50≤50
3	50＜LD50≤300
4	300＜LD50≤2000
5	5000

Import and distribution for Serbia: Farmadria DOO
info@avenalab.com
+381 (0) 69 / 55 65 029
www.avenalab.com

EFFICACY TEST

5. Testing results

Table 1 Cell viability under different concentrations of test substances

Concentrations (mg/mL)	Control	7	6	5	4	3
Cell viability (%)	100±2.86	17.73±2.25	29.95±1.67	44.40±2.58	62.08±3.33	84.87±2.61

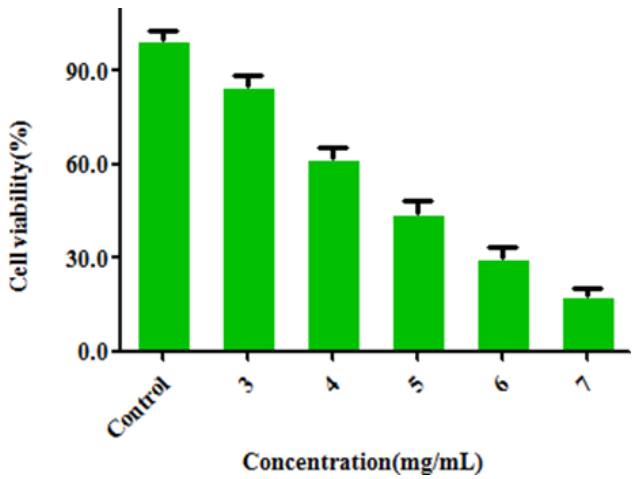

Fig.1 Cell viability under different concentrations of Sodium ascorbyl phosphate

6. Testing conclusions

According to SN/T 2328-2009 and MTT method, the IC50 of Vitamin C (Sodium ascorbyl phosphate) is 4.66mg/mL on the NIH/3T3 cell line. Sodium ascorbyl phosphate had no toxicity on NIH/3T3 cell line when the concentration was not more than 3.62mg/mL. The acute oral toxicity LD50 of sodium ascorbyl phosphate was 2447mg/kg (rat), so sodium ascorbyl phosphate was classified as grade 5 (non-toxic) according to GHS acute toxicity classification standard.