Report No.: BP-S-25013885-C1EN

Test Report

Sample Name:	PLGA
Client Name:	eSUNMed Biotechnology (Shenzhen) Co., Ltd.
Client Address:	3F, No. 9, Yifeng Hua Innovation Industrial Park, Xinshi Community, Dalang Street, Longhua District, Shenzhen
Test Items:	MTT cytotoxicity test
Date of Issue:	2025.04.17

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DECLARE

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Organization name: **Shanghai WEIPU Testing Technology Group Co., LTD.**

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Task No.	/	Detection category	Commission test
Sample No.	BP-S-25013885	Sample source	Sent by client
Sample name	PLGA	Batch number	PLGA50502411271 0011100
Specification	50:50	Sample quantity	1pc
Model	/		
Manufacturer	eSUNMed Biotechnology (Shenzhen) Co., Ltd. Wuhan branch office		
Manufacturer address	Room 401, Building 13, Block B, Donghu Hi-tech International Health City, No. 24, Gold-Industrial-Park Road, Zhengdian Street, Jiangxia District, Wuhan		
Client	eSUNMed Biotechnology (Shenzhen) Co., Ltd.		
Client address	3F, No. 9, Yifeng Hua Innovation Industrial Park, Xinshi Community, Dalang Street, Longhua District, Shenzhen		
Receiving date	2025.03.05		
Test location	3rd Floor, Building 7,166-1, Fengjin Road, Fengxian District, Shanghai.		
Test period	2025.03.05 to 2025.03.18		
Test item	MTT cytotoxicity test		
Test criterion	ISO 10993-5:2009		
Test conclusion	The cell viability of 100% test sample extract is 106.16%, and the cell morphology grade is 0, the sample extract had no potential toxic effect on L-929 cells. Date of issue: 2025.04.17		
Implementation standard	ISO/IEC 17025: 2017; RB/T214—2017		
Remarks	"/" in the report indicates that this item is blank		

Edited by	Checked by	Approved by (Authorized signatory)
Date: 2025.04.17	Date: 2025.04.17	Date: 2025.04.17

Catalogue

1 Objective

The biological response to L-929 cells was evaluated by in vitro cytotoxicity test.

2 Test method

MTT cytotoxicity test

3 Test and control samples

3.1 Test samples

The information in the form is provided by the client.

Sample name	PLGA
Sterilization state	Unsterilized
Sterilization methods	/
Sample material	PLGA
Application	/

3.2 Control samples

Negative control sample：HDPE	
Manufacturer	USP
Specification	Three-piece pack
Batch No.	R149K0
Positive control sample：DMSO	
Manufacturer	Sinopharm Chemical Reagent Co., Ltd.
Specification	500mL/bottle
Batch No.	20230922
Blank control sample：The MEM medium contained 10% FBS	

4 Reagents and Instrument

4.1 Reagents

Name	Supplier
FBS	Bio-Channel
1×MEM medium (100IU/mL PNC，100μg/mL Streptomycin)	Bio-Channel
Trypsin (EDTA) solution	Gibco
PBS	Biosharp

Name	Supplier
MTT	Beyotime
IPA	Sinopharm Chemical Reagent Co., Ltd.

4.2 **Instrument**

Name	Instrument ID	Calibration is valid until
Clean bench	WPE-TL0125	2025.10.09
Electronic scales	WPE-TL0242	2025.04.10
Double plate constant temperature oscillation incubator	WPE-TL0390	2025.10.09
Biological microscope	WPE-TL0139	/
Centrifuge	WPE-TL0279	2025.04.10
Microplate reader	WPE-TL0293	2025.10.09
CO_2 incubator	WPE-TL0077	2025.04.10
pH meter	WPE-TL0394	2025.11.20
Steel ruler	WPE-TL0033	2025.07.18

5 Test system

Cloning L929 is standard recommended cell line, and this cell comes from Cell Bank/Stem Cell Bank, Chinese Academy of Sciences.

Contact of the test sample with the test system via an extract solution (The MEM medium contained 10% FBS) is considered the optimal route of administration and is the recommended method in standard.

6 Experimental content

6.1 Sterilization

Petri dishes, porous culture plates, pipette tips, and other utensils that may be used in the test are sterilized by high pressure steam prior to the test.

6.2 Sample preparation

Under aseptic operation, the extracts were prepared according to the method in the table below. After the extraction, the changes of the extracts were checked. The extracts were not centrifuged and etc. The pH was not adjusted. Blank control, negative control and positive control samples were prepared by the same method.

Table 6-1 Preparation of extracts

Extraction solvent	Actually sample	Sampling ratio	Solvent volume	Sampling condition	Whether it is clear	pH
MEM medium containing 10%FBS	2.0129g	0.2g:1mL	13.13mL[a]	37℃ 24h 60rpm	Yes	8.00

a: Sample absorption rate is 1.53mL/g.

6.3 Test procedure

The test procedure is sterile operation.

L929 monolayer cells cultured in 10% FBS MEM medium for 48 h to 72 h were liquefied with enzyme liquid (trypsin / EDTA).

The cells are then resuspended in culture medium and the cell suspension is adjusted at a density of 1×10^5 cells/mL.

Using a multichannel pipette, dispense 100 µl culture medium only (blank) into the peripheral wells of a 96-well tissue culture microtitre plate. In the remaining wells, dispense 100 µl of a cell suspension of 1×10^5 cells/mL. Set blank (left and right 2 groups), negative control, positive control, sample group, each group has 6 parallel wells.

Incubate cells for 24 h (5% CO_2, 37℃, > 90% humidity) so that cells form a half-confluent monolayer.

After 24 h incubation, aspirate culture medium from the cells. Dispense 100µL of the test solution, including the sample at the appropriate concentration, negative control, positive control, and blank control, into each well. Four different test sample concentrations (100%, 50%, 25%, 12.5%) were tested.

Incubate cells for 24 h (5% CO_2, 37℃, > 90% humidity).

After 24h of testing, the plate and cell morphology were examined under an inverted biomicroscope, and the changes in cell morphology due to cytotoxicity of the sample extract was recorded.

After the examination of the plates, carefully remove the culture medium from the plates. 50 µl of the MTT solution is then added to each test well and the plates are further incubated for 2 h in the incubator at 37°C. Then the MTT solution is discarded and 100 µl of isopropanol are added in each well. Sway this plate and subsequently transfer it to a microplate reader equipped with a 570nm filter to read the absorbance (reference wavelength 650nm).

6.4 Data analysis

Compared with blank group, cell survival rate was calculated by following formula.

$$\text{Viab.}\ (\%) = \frac{100 \times OD_{570e}}{OD_{570b}}$$

where: OD_{570e}——is the mean value of the measured optical density of the 100 % extracts of the test sample.

OD_{570b}——is the mean value of the measured optical density of the blanks.

6.5 Microscope evaluation

According to standard, A useful way to grade test samples is given in Table 6-2.

Table 6-2 Qualitative morphological grading of cytotoxicity of extracts

Grade	Reactivity	Conditions of all cultures
0	None	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth
1	Slight	Not more than 20% of the cells are round, loosely attached and without intracytoplasmatic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmatic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cell layers not completly destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

6.6 Quality check

A test meets the acceptance criteria if the mean OD_{570} of blanks is ≥ 0.2.

A test meets acceptance criteria if the 96-well plate left side (row 2) and the right side (row 11) mean of the blanks do not differ by more than 15 % from the mean of all blanks.

6.7 Evaluation criteria

The lower the Viab. % value, the higher the cytotoxic potential of the test item is.

If viability is reduced to < 70% of the blank, it has a cytotoxic potential. The 50% extract of the test sample should have at least the same or a higher viability than the 100% extract; otherwise the test should be repeated.

7 Test result

The qualitative morphological classification of cytotoxicity of extracts from different groups was shown in Table 7-1. The result of cell viability (%) for the test sample extracts at concentrations of 100%, 50%, 25%, and 12.5% was shown in Table 7-2

Table 7-1 Qualitative morphological classification of cytotoxicity of extracts from different groups

Group	Cell morphology observation	Grade
Blank control	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth	0
Positive control	Nearly complete or complete destruction of the cell layers.	4
Negative control	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth	0
Sample solution (100%)	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth	0
Sample solution (50%)	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth	0
Sample solution (25%)	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth	0
Sample solution (12.5%)	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth	0

Table 7-2 Optical Density and Viability

Group	Sample solution (100%)	Sample solution (50%)	Sample solution (25%)	Sample solution (12.5%)	Negative control	Positive control	Blank control
Average value	0.4553	0.4558	0.4745	0.4397	0.4642	0.1288	0.4289
SD	0.0351	0.0310	0.0139	0.0282	0.0462	0.0100	0.0395
Survival rate %	106.16	106.28	110.63	102.51	108.22	30.04	100.00

8 Deviations

The test was carried out in strict accordance with the standard operating procedures, and no deviation affecting the validity of the test data occurred.

9 Record Preservation

All raw data and records related to this test and copies of the final report are kept in the archives.

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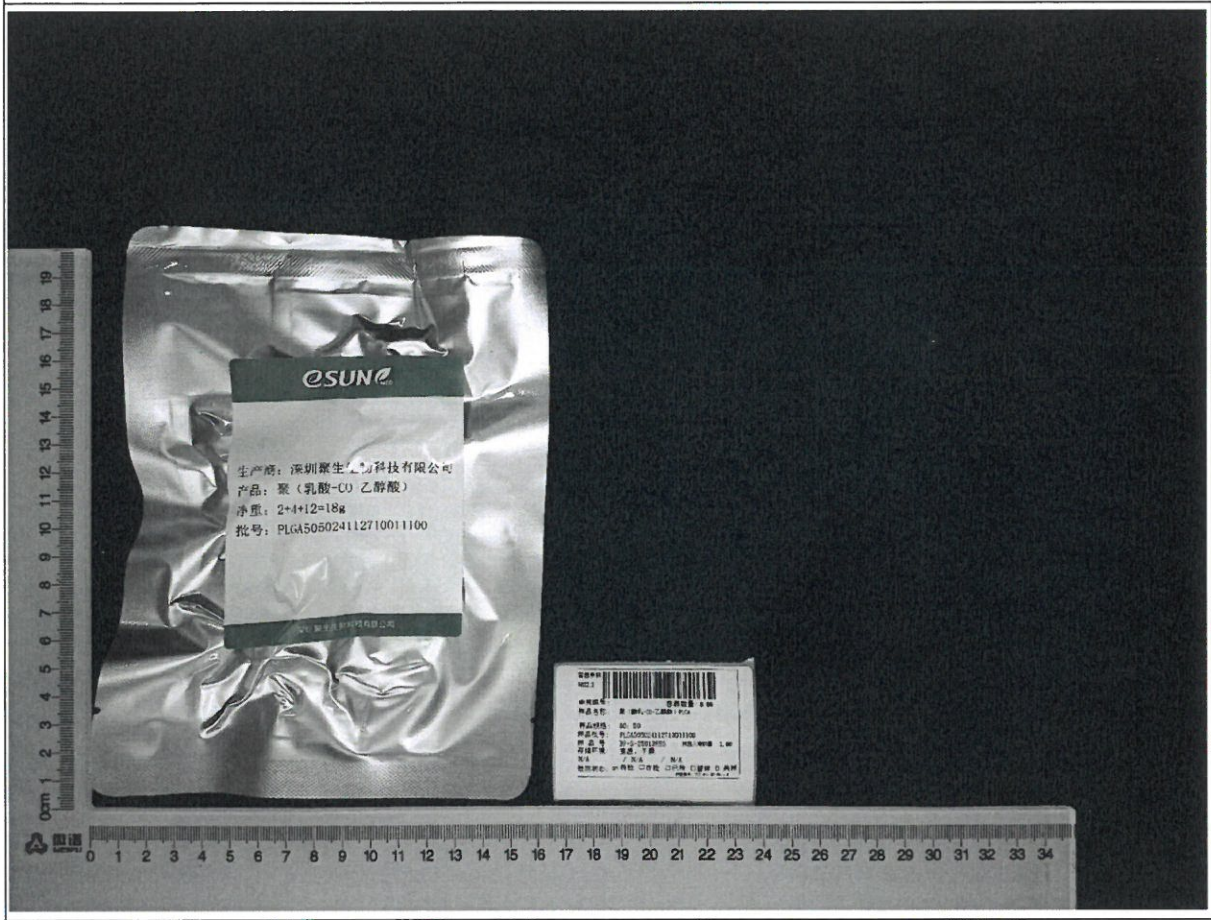
Test report photo page

Photos and descriptions

Test component description
Random sampling
Model, specification or other description
/

***** End of report *****