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CNAS L9334

微谱 WEIPU

Report No: BP-A-2335534EN

Test Report

Sample Name:	FEP Heat Shrink Tube
Client Name:	Forbest Manufacturing Co Ltd.
Client Address:	Fulian industrial Longhua Town Shenzhen City Guangdong Province China
Test item	MTT cytotoxicity test
Date of Issue:	2024.01.09

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DECLARE

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Task No.	BP-A-2335534	Detection category	Commission test
Sample No.	BP-S-23093129	Sample source	Sent by client
Sample name	FEP Heat Shrink Tube	Batch number	/
Model	4.9mm*0.25mm*100mm	Sample number	2
Specification	/		
Manufacturer	/		
Manufacturer address	/		
Client	Forbest Manufacturing Co Ltd.		
Client address	Fulian industrial Longhua Town Shenzhen City Guangdong Province China		
Receiving date	2023.11.21		
Test location	3 Floor, Building 7,166-1, Fengjin Road, Fengxian District, Shanghai.		
Test period	2023.11.21 to 2023.11.29		
Test item	MTT cytotoxicity test		
Test criterion	GB/T 16886.5-2017/ISO 10993-5:2009		
Test conclusion	The cell viability of test sample extract was 76.55%, and the cell morphology grade of test sample extract was grade 1, the sample extract had no potential toxic effect on L-929 cells. Date of issue 2024.01.09		
Implementation standard	ISO/IEC 17025:2017; RB/T214—2017		
Remarks	"N/A" in the report indicates that this item is not applicable, and "/" in the report indicates that this item is blank.		

Edited by	Checked by	Approved by (Authorized signatory)
郭桂芳	王菲	
Date: 2024.01.09	Date: 2024.01.09	Date: 2024.01.09

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 conclusion

The cell viability of test sample extract was 76.55%, and the cell morphology grade of test sample extract was grade 1, the sample extract had no potential toxic effect on L-929 cells.

4 Test and control samples

4.1 Test samples

The information in the form is provided by the client

Sample name	FEP Heat Shrink Tube
Sterilization state	Unsterilized
Sterilization methods	/
Sample material	/
Physical condition	Solid
Color	See photo page of inspection report for details
Preservation conditions	Room temperature, dry
Application	/

4.2 Control samples

Negative control sample: HDPE	
Manufacturer	USP
Specification	Three-piece pack
Batch No.	R149K0
Characteristics	Solid
Color	White
Preservation condition	Room temperature
Positive control sample: DMSO	
Manufacturer	Sinopharm Chemical Reagent Co., Ltd.
Specification	500mL/bottle
Batch No.	20230324

Characteristics	Liquid
Color	Colorless
Preservation condition	Room temperature
Blank control sample：The MEM medium contained 10% FBS	
Characteristics	Liquid
Color	Pink
Preservation condition	4℃

5 Reagents and Instrument

5.1 Reagents

Name	Supplier
FBS	damas life®
MEM medium (100IU/mLPNC，100ug Streptomycin)	Bio-Channel
Trypsin (EDTA) solution	Gibco
PBS	Biosharp
MTT	Beyotime
IPA	Sinopharm Chemical Reagent Co., Ltd.

5.2 Instrument

Name	Instrument ID
CO_2 incubator	WPE-TL0077
Biological microscope	WPE-TL0139
Clean bench	WPE-TL0125
Centrifuge	WPE-TL0279
Constant temperature incubator	WPE-TL0275
Electronic scales	WPE-TL0242
ELISA Reader	WPE-TL0170
pH meter	WPE-TL0079

6 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.

7 Test procedure

7.1 Sterilization

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

7.2 Sample preparation

The samples were irradiated with ultraviolet rays before dipping and sterilized for 30min.

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 7-1 Preparation of extracts

Extraction solvent	Actually sample	Sampling ratio	Solvent volume	Sampling condition	Whether the extract is clear	pH
MEM medium containing 10%FBS	32.342cm^2	6cm^2:1mL	5.39mL	37±1℃ 24±2h 60rpm	Yes	8.82

7.3 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 °C, 90 % humidity) so that cells form a half-confluent monolayer.

After 24 h incubation, aspirate culture medium from the cells. Per well, add 100 µl of treatment medium containing either the appropriate concentration of sample extract, or the negative control, or the positive control, or blank control. Four different sample extraction concentrations (100%, 50%, 25%, 12.5%) were tested.

Incubate cells for 24 h (5 % CO_2, 37 °C, 90 % humidity).

After 24h of testing, the plate and cell morphology were examined under an inverted biommicroscope, 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 discanted 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).

7.4 Data analysis

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

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

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.

7.5 Qualitative evaluation

According to standard, A useful way to grade test samples is given in Table 7-2.

Table 7-2 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.

7.6 Quality check

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.

7.7 Presentation of results

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 vv; otherwise the test should be repeated.

8 Test result

The qualitative morphological classification of cytotoxicity of extracts from different groups was shown in Table 8-1. Viab. (100%) was shown in Table 8-2.

Table 8-1 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%)	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.	1
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 8-2 Optical Density and Viab.%

Group	Average Optical Density	Viab.(%)
Blank control	0.5231±0.0154	100
Positive control	0.1446±0.0078	27.65
Negative control	0.6010±0.0376	114.87
Sample solution (100%)	0.4005±0.0221	76.55
Sample solution (50%)	0.5400±0.0274	103.21
Sample solution (25%)	0.5754±0.0215	109.59

Group	Average Optical Density	Viab.(%)
Sample solution (12.5%)	0.5998±0.0401	114.65

9 Test conclusion

The cell viability of test sample extract was 76.55% the cell morphology grade of test sample extract was grade 1, the sample extract had no potential toxic effect on L-929 cells.

10 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.

11 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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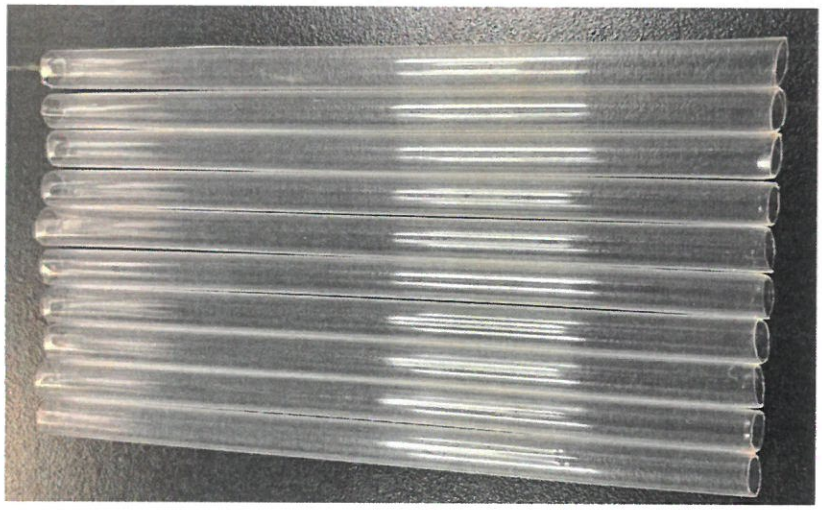
Test report photo page

Photos and descriptions
 FIG. 1 Detailed diagram
 FIG. 2 Sample enlargement
Test component description
Overall sampling
Model, specification or other description
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***** End of report *****