

功效检测报告

EFFICACY TEST EPORT

报告编号 Report No: RG240337-6CE

客户名称 The name of the customer: 聚磷锡创生物科技（无锡）有限公司
Polyphosphate Xichuang Biotechnology (Wuxi)
Co., Ltd.

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样品名称 Sample name: 多聚磷酸钠（PolyP45）

申请项目 Test Item: 舒缓 soothing

报告发布日期 Date for 2024.11.25

Reporting:

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广州华测品鉴生物技术有限公司

CTI Pinjian Biotechnology (Guangzhou) Co.Ltd.

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检测结果
Test results

样品信息 Sample information	样品名称 Name of Sample	多聚磷酸钠 (PolyP45)	样品来源 Sample Source	客户送检 The customer sends the sample
	样品规格 Sample Specification	100 g	批号 Sample Batch	N/A
	生产日期 Production date	N/A	保质期/限期使用日期 Shelf life/expiration date	N/A
	样品性状 Sample Properties	固体粉末 Solid powder	样品保存条件 Save the condition	4 度保存 Save at 4 degrees
	生产厂家 Manufact	聚磷锡创生物科技（无锡）有限公司 Polyphosphate Xichuang Biotechnology (Wuxi) Co., Ltd.		
	生产厂家地址 Manufacturer's address	无锡市锡山区安镇街道东安路 88 号 1 层 103B 103B, 1st Floor, No. 88 Dong'an Road, Anzhen Street, Xishan District, Wuxi City		
	其他信息 Additional Information	N/A		
客户信息 Customer Information	客户名称 The name of the customer	聚磷锡创生物科技（无锡）有限公司 Polyphosphate Xichuang Biotechnology (Wuxi) Co., Ltd.		
	客户联络信息 Customer contact information	无锡市锡山区安镇街道东安路 88 号 1 层 103B 103B, 1st Floor, No. 88 Dong'an Road, Anzhen Street, Xishan District, Wuxi City		
样品来源为客户送检时，以上信息由客户提供，我司不对信息的有效性和真实性负责。 When the sample source is sent by the customer for inspection, the above information is provided by the customer, and our company is not responsible for the validity and authenticity of the information.				
表 1 检测信息 Table 1 Detection information				
样品接收日期 Sample received Date	2024.08.22		测试实施日期 Test Date	2024.09.06-2024.09.23
检测地址 Detection address	广州市黄埔区博华一路 203 号 B1201 室 Room B1201, No. 203, Bohua 1st Road, Huangpu District,Guangzhou			
表 2 检测结果 Table 2 Test results				

样品号 Sample No.	测试项目 Test Item	测试方法 Test Method	测试结果 Result	判定规则 Judgment Rules	测试结论 Conclusion
G240337-1	舒缓 soothing	WI-GZB-737 化妆品体外功效测试-HGF细胞炎症因子含量测定* WI-GZB-737 Cosmetic in vitro efficacy test-HGF cell inflammatory factor content determination *	样品在 0.1%测试浓度下, TNF-α抑制率=32.64%样品; 在 0.01%测试浓度下, TNF-α抑制率=24.92%; 样品在 0.001%测试浓度下, TNF-α抑制率=22.61% The inhibition rate of TNF-α was 32.64% at the concentration of 0.1%, The inhibition rate of TNF-α was 24.92% at the concentration of 0.01%, The inhibition rate of TNF-α was 22.61% at 0.001% test concentration	见附件: 8 结果有效性 See Annex 8: Result validity	在本次实验条件下, 0.1%、0.01%、0.001%浓度的样品具有舒缓的效果。 Under the experimental conditions, 0.1%, 0.01% and 0.001% of the samples has soothing effect.

备注: 1.具体测试数据见附件; 2.*测试方法为实验室方法。

Remarks: 1. See the attachment for specific test data;2. *The test method is a laboratory method.

附件 Annex

1. 仪器 Instrument

倒置显微镜、酶标仪、二氧化碳培养箱、离心机、细胞计数仪、生物安全柜。

Inverted microscope, microplate reader, carbon dioxide incubator, centrifuge, cell counter, biological safety cabinet.

2. 实验材料 Experimental materials

完全培养基 Complete medium	DMEM 高糖培养基+15%血清 DMEM high glucose medium + 15% serum
MTT 工作液 MTT working solution	用完全培养基将 MTT 稀释为 1mg/mL MTT 工作液，现配现用。 MTT was diluted to 1 mg/mL MTT working solution with complete culture medium, which was prepared and used immediately.
样品 Sample	将样品稀释成 0.5%、0.2%、0.1%、0.05%、0.01%五个浓度梯度进行细胞毒性实验，筛选 0.1%、0.01%、0.001%三个浓度进行功效测试实验。 The samples were diluted into five concentrations of 0.5%, 0.2%, 0.1%, 0.05% and 0.01% for cytotoxicity test, and then three concentrations gradients of 0.1%, 0.01% and 0.001% were selected for subsequent efficacy test.
阳性对照 Positive control	地塞米松 Dexamethasone.
细胞及代次 Cells and generations	牙龈成纤维细胞（HGF 细胞）购自北京博瑞康沐生物，毒性测试为 9 代，功效测试为 10 代。 Gingival fibroblasts (HGF cells) were purchased from Beijing Borui Kangmu Biology. The toxicity test was 9 generations and the efficacy test was 10 generations.

3. 试验原理 Test principle

细菌及其产物是牙周炎的主要始动因子，牙龈卟啉单胞菌（P.g）作为细菌红色复合体中最具代表性的一员，在牙周感染位点尤其是深牙周袋中含量升高而被广泛研究。P.g 的致病原包括牙龈素、脂多糖等，脂多糖作为革兰阴性细菌细胞外膜上一类最主要的大分子抗原，因其对牙周组织极高的毒性，而成为一类关键的毒力因子。牙周组织的破坏包括牙槽骨和牙周软组织的破坏，主要由炎症介质包括如白介素 6（IL-6）和前列腺素 E2(PGE2)，及组织破坏性酶包括如 MMP-1 等介导。而肿瘤坏死因子-α（TNF-α）等初级炎症介质可以诱导趋化因子向牙周组织聚集，加重结缔组织的破坏和破骨细胞性骨吸收。通过测定给药后样品组的炎症因子抑制率，评价样品对炎症因子释放的抑制作用。

Bacteria and their products are the main initiating factors of periodontitis. Porphyromonas gingivalis (P. G), as the most representative member of the bacterial red complex, has been widely studied because of

its increased content in periodontal infection sites, especially in deep periodontal pockets. The pathogens of P. G include gingivitin, lipopolysaccharide and so on. Lipopolysaccharide, as one of the most important macromolecular antigens on the outer membrane of Gram-negative bacteria, is a key virulence factor because of its high toxicity to periodontal tissue. Periodontal tissue destruction, including destruction of alveolar bone and periodontal soft tissue, is mediated by inflammatory mediators such as interleukin 6 (IL-6) and prostaglandin E2 (PGE2), and tissue damaging enzymes such as MMP-1. Tumor necrosis factor- α (TNF- α) and other primary inflammatory mediators can induce chemokines to accumulate in periodontal tissue, aggravate the destruction of connective tissue and osteoclastic bone resorption. The inhibitory effect of the sample on the release of inflammatory factors was evaluated by measuring the inhibitory rate of inflammatory factors in the sample group after administration.

4. 测试分组 Test Grouping

组别 Group	诱导条件 Induction conditions	培养条件 Culture conditions
空白组 Blank group	/	完全培养基 Complete medium
模型组 Model Group	LPS	完全培养基 Complete medium
阳性对照组 Positive control group	LPS	含地塞米松完全培养基 Dexamethasone-containing complete medium
受试样品组 Test sample group	LPS	含样品的完全培养基 Complete medium with sampled

5. 实验步骤 Experimental steps

5.1. 细胞传代培养 Cell subculture

参照 WI-GZB-703 规程进行细胞的复苏、培养。

Perform cell recovery and culture according to the WI-GZB-703 cell recovery, passage, and cryopreservation protocol..

5.2. 细胞毒性的测定 Determination of cell activity

参照 WI-GZB-717 规程进行细胞毒性实验，根据细胞毒性结果选择合适浓度的样品进行后续功效实验。

The cytotoxicity test was carried out according to the WI-GZB-717 procedure, and the samples with appropriate concentration were selected for the subsequent efficacy test according to the cytotoxicity

results.

5.3. 种板 Cell seed plate

选择对数生长期的细胞, 经胰酶消化后收集细胞, 制成细胞悬液并计数。用细胞培养基稀释细胞至合适接种密度, 接种至 96 孔板中, 每孔 100 μ L。接种结束后, 放置于 CO₂ 培养箱中培养 24h $\pm$ 2h。

Cells in logarithmic growth phase were selected and digested by trypsin, then the cells were collected, made into cell suspension and counted. Cells were diluted with cell culture medium to an appropriate seeding density and seeded into 96-well plates at 100 μ L per well. After inoculation, the cells were incubated in a CO₂ incubator for 24 $\pm$ 2 hours..

5.4. 给药 Drug administration

弃掉 96 孔板中的培养基, 开展诱导及给药操作。受试物孔中加入含有一定浓度受试物和 LPS 的培养基, 模型孔加入含有 LPS 的培养基, 阳性对照孔中加入含有阳性对照和 LPS 的培养基, 空白对照孔中加入正常的细胞培养基, 每孔 200 μ L。将 96 孔板置于 CO₂ 培养箱中培养 24h $\pm$ 2h。

The culture medium in the 96-well plate was discarded, and induction and administration operations were carried out. A culture medium containing a certain concentration of test substance and LPS was added to the test sample hole, a culture medium containing LPS was added to the model hole, a culture medium containing positive control and LPS was added to the positive control hole, and a normal cell culture medium was added to the blank control hole, 200 μ L per hole. The 96-well plates were incubated in a CO₂ incubator for 24 $\pm$ 2 hours.

5.5. 细胞上清收集 Cell supernatant was collected

孵育培养结束后, 收集细胞培养液于无菌离心管中, 离心后收集细胞培养上清液, 置于-80 °C 超低温冰箱冷冻保存备用。

After the incubation and culture, the cell culture solution was collected in a sterile centrifuge tube, and the supernatant of cell culture was collected after centrifugation and stored in an ultra-low temperature refrigerator at -80°C for later use.

5.6. ELISA 检测 ELISA test

ELISA 检测需根据体外炎症因子含量测定试剂盒的使用说明书进行检测。

ELISA detection should be carried out according to the instructions for use of the kit for determining the content of inflammatory factors in vitro.

6. 数据分析 Data analysis

6.1. 细胞毒性计算 Cytotoxicity calculations

细胞活性 (%) = (样品孔_{OD} - 调零孔_{OD}) / (空白对照孔_{OD} - 调零孔_{OD}) * 100%

Cell activity (%) = (sample well OD - zero setting OD value) / (blank control well OD - zero setting OD value) * 100%

6.2. 炎症因子含量抑制率的计算公式 Formula for calculating the inhibition rate of inflammatory factor content

抑制率 (%) = (1 - T/C) × 100%

Inhibition rate (%) = (1 - T/C) × 100%

式中:

Where:

T—受试物炎症因子含量平均值;

T — mean value of inflammatory factor content of the test substance;

C—模型组炎症因子含量平均值。

C — Mean value of inflammatory factors in the model group.

6.3. 炎症因子相对含量的计算公式 Calculation formula of relative content of inflammatory factor

相对含量 (%) = (T/C) × 100%

Relative content (%) = (T/C) × 100%

式中:

Where:

T—受试物炎症因子含量平均值;

T — mean value of inflammatory factor content of the test substance;

C—模型组炎症因子含量平均值。

C — Mean value of inflammatory factors in the model group.

7. 测试有效性判定条件 Test validity determination condition

7.1. 细胞毒性 Cytotoxicity

每组平行复孔 OD 值的变异系数 (Coefficient of Variation, C.V), C.V 值 ≤ 20%。

The coefficient of variation (C.V) of absorbance OD value of three biological replicates in each group was ≤ 20%.

7.2. 炎症因子含量 Inflammatory factor content

每批次试验应设置模型组及阳性对照, 要求每批次模型组与空白对照相比, 炎症因子含量显著性升高 ($P < 0.05$) 或模型组与空白对照相比, TNF- α 含量上调 20%; 阳性对照与模型组相比, 炎症因子含量显著性降低 ($P < 0.05$) 或阳性对照与模型组相比抑制率 $\geq 20\%$, 则认为试验系统有效。

A model group and a positive control should be set up for each batch of tests, and the content of inflammatory factors in the model group was required to be significantly increased ($P < 0.05$) compared with the blank control, or the content of TNF- α in the model group was required to be increased by 20% compared with the blank control. If the content of inflammatory factors in the positive control was significantly lower than that in the model group ($P < 0.05$) or the inhibition rate of the positive control was more than or equal to 20%, the test system was considered to be effective.

统计各组平行孔间吸光度的标准差 (Standard Deviation, SD), 并计算变异系数 (Coefficient of Variation, C.V), C.V 值 $\leq 20\%$, 则认为试验平行性有效。

The Standard Deviation (SD) of absorbance between parallel wells in each group was counted, and the coefficient of variation (Coefficient of Variation, C. V) was calculated. If the C. V value was $\leq 20\%$, the test parallelism was considered to be valid.

8. 结果有效性 Result validity

样品与模型组相比炎症因子 TNF- α 含量具有显著性降低 ($P < 0.05$), 则认为样品具有抑制炎症因子 TNF- α 的作用。

Compared with the model group, the content of inflammatory factors TNF- α in the sample was significantly reduced ($P < 0.05$), and it was considered that the sample had the effect of inhibiting inflammatory factor TNF- α .

9. 测试结果 Test results

9.1. 细胞毒性测试结果 Cytotoxicity Test Result

如表 1 和图 1 所示, 样品组和空白对照组 (BC) 的吸光度 OD 值变异系数 C.V 值均小于等于 20%, 试验有效。

As shown in Table 1 and Figure 1, the coefficient of variation (C.V) of the absorbance OD value of the sample group and the blank control group (BC) is less than or equal to 20%, and the test is valid.

样品在 0.1%、0.05%、0.01% 这三个测试浓度下, 与细胞共培养后细胞活性均大于等于 85%, 表明样品在 0.1% 及以下的测试浓度均可用于后续细胞功效测试实验。

Under the three test concentrations of 0.1%, 0.05% and 0.01%, the cell activity of the sample after being co-cultured with cells is greater than or equal to 85%, which indicates that the test concentration of the sample at or below 0.1% can be used for subsequent cell efficacy test experiments.

表 1 不同浓度样品的细胞活性 (Mean±SD)

Table 1 Cell activity of samples with different concentrations (Mean ± SD)

实验组别 Experimental group	细胞活性 (%) Cell activity (%)	OD 值 C.V 值 (%) OD value C. V value (%)
BC	100.00±0.41	0.34
0.5%-样品 0.5%-Samples	7.58±2.26	7.96
0.2%-样品 0.2%-Samples	6.63±1.48	5.38
0.1%-样品 0.1%-Samples	93.39±1.88	1.65
0.05%-样品 0.05%-Samples	95.72±0.30	0.26
0.01%-样品 0.01%-Samples	93.56±2.99	2.62

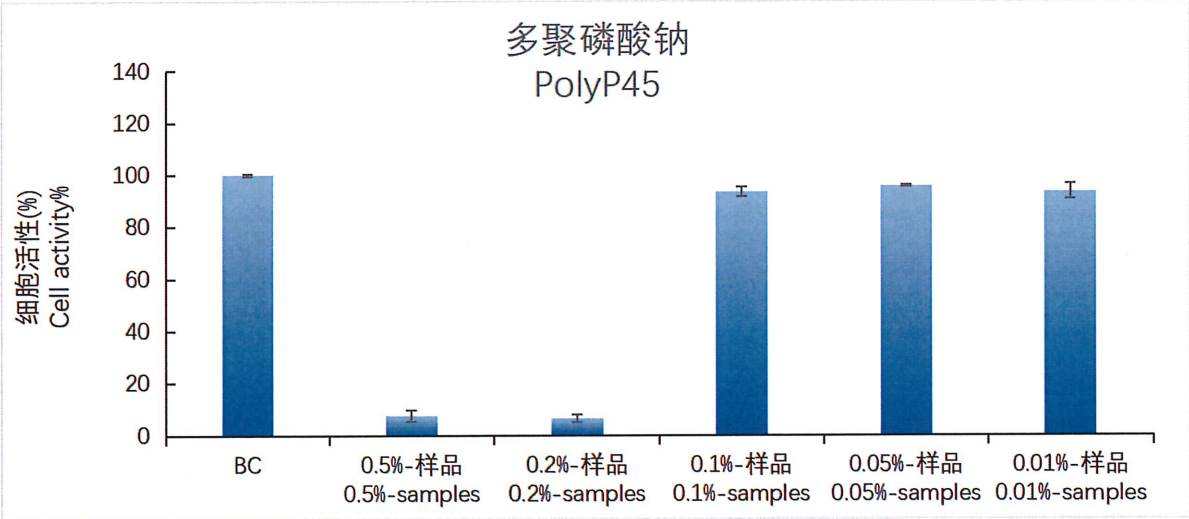


图 1.不同浓度样品的细胞活性 (Mean±SD)

Fig. 1. Cell activity of samples at different concentrations (Mean ± SD)

结合细胞毒性结果和实验需求，最终选择 0.1%、0.01%、0.001%浓度的样品进行后续功效测试。

Based on the results of cytotoxicity and experimental requirements, samples with concentrations of 0.1%, 0.01% and 0.001% were finally selected for subsequent efficacy tests.

9.2. 体外炎症因子 TNF-α含量测定结果 Determination of inflammatory factor TNF-α in vitro

模型组 (M) 与空白对照组 (BC) 相比，体外炎症因子 TNF-α含量显著性升高 (P<0.05)，

阳性对照组 (PC) 与模型组 (M) 相比，体外炎症因子 TNF-α含量显著性降低 (P<0.05)，且每组

OD 值的变异系数 C.V 值≤20%，试验系统有效。样品在 0.1%、0.01%、0.001%测试浓度时，细胞中的炎症因子 TNF-α相对含量分别为 67.36%、75.08%、77.39%，与模型组（M）相比，TNF-α含量显著降低，具体数据见表 2 和图 2 所示。

Compared with the blank control group (BC), the content of TNF-α in vitro in the model group (M) was significantly increased ($P < 0.05$). Compared with the model group (M), the positive control group (PC) showed a significant decrease in the content of in vitro inflammatory factor TNF-α ($P < 0.05$), and the coefficient of variation (C.V) of the OD value of each group was $\leq 20\%$, indicating that the test system was effective. The relative content of inflammatory factor TNF-α in the cells was 67.36%, 75.08% and 77.39% respectively at the test concentrations of 0.1%, 0.01% and 0.001%, and the content of TNF-α was significantly lower than that in the model group (M), as shown in Table 2 and Figure 2.

表 2 不同组别细胞 TNF-α相对含量（Mean±SD）及抑制率
（*表示与模型组相比，差异具有统计学意义，* $P < 0.05$ ，** $P < 0.01$ ）

Table 2 Relative content of TNF-α in different groups of cells (Mean ± SD) and inhibition rate
(* indicates a statistically significant difference compared to the model group, * $P < 0.05$, ** $P < 0.01$)

测试组别 Test group	TNF-α相对含量（Mean±SD）% Relative content of TNF-α (Mean ± SD)%	TNF-α抑制率% TNF-α inhibition rate%
空白组（BC） Blank group (BC)	66.96±3.43**	N/A
模型组（M） Model Group	100.00±5.83	N/A
阳性对照（PC） Positive Control (PC)	65.99±1.60**	34.01
0.1%-样品 0.1%-Samples	67.36±3.16**	32.64
0.01%-样品 0.01%-Samples	75.08±3.47**	24.92
0.001%-样品 0.001%-Samples	77.39±5.20*	22.61

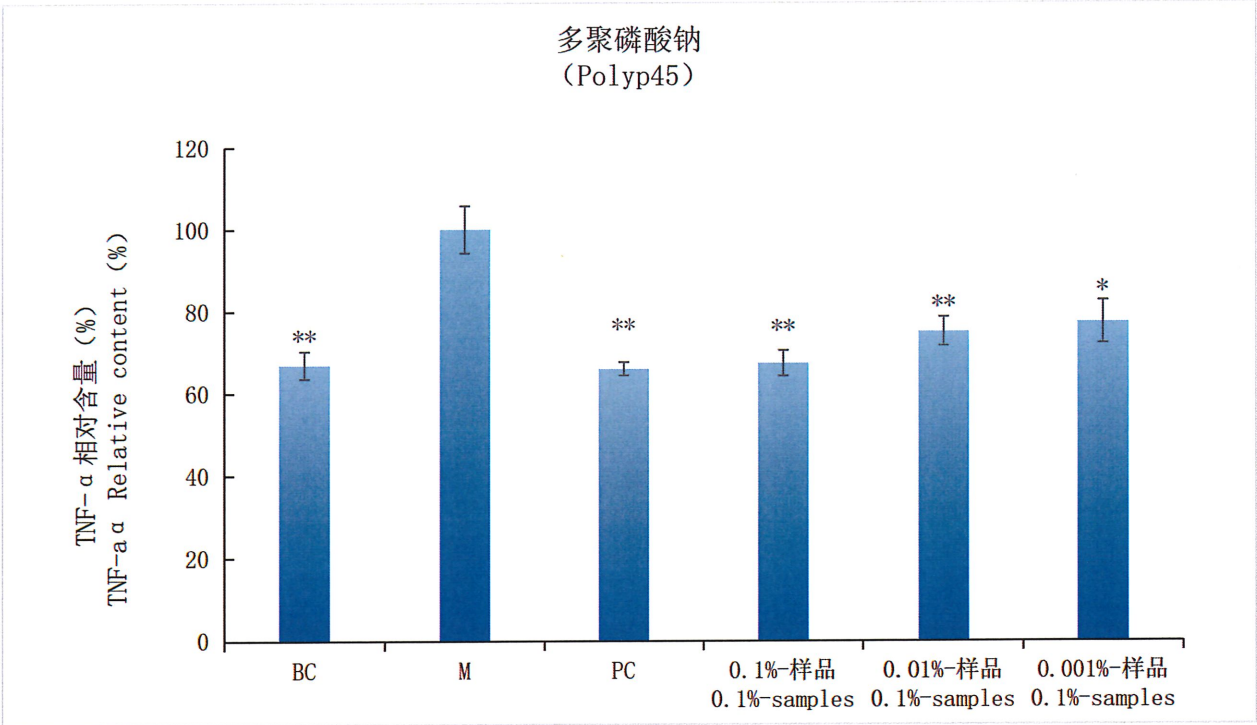


图 2 不同组别细胞 TNF-α相对含量 (Mean±SD)

备注: *表示与模型组相比, 差异具有统计学意义 (* $P<0.05$, ** $P<0.01$)。

Figure 2 Relative content of TNF-α in different groups of cells (Mean ± SD)

Note: * means that the difference is statistically significant compared with the model group (* $P < 0.05$, ** $P < 0.01$).

10. 结论 Conclusion

在本次试验条件下, 样品“多聚磷酸钠 (PolyP45)”在 0.1%、0.01%、0.001%测试浓度时, 细胞中的炎症因子 TNF-α抑制率分别为 32.64%、24.92%、22.61%, 表明 0.1%、0.01%、0.001%浓度的样品对炎症因子 TNF-α有抑制作用。

Under the condition of this experiment, when the concentration of the sample "PolyP45" is 0.1%, 0.01% and 0.001%, the inhibition rate of the inflammatory factor TNF-alpha in the cells is 32.64%, 24.92% and 22.61% respectively, The results showed that 0.1%, 0.01% and 0.001% of the samples had inhibitory effect on inflammatory factor TNF-α.

11. 备注: Remark:

a) 本报告中的测试结果和结论仅适用于该客户提供的该批样品。因此, 该报告中的结果并不代表除该样品以外的样本或类似样品;

The testing results and conclusions in this report are only applicable to the batch of samples provided by the customer. Therefore the results in this report do not represent samples other than this one or similar samples.

b) 本报告中的数据结果仅供科研、教学、企业内部质量控制、企业内部产品研发等目的用。

The data results in this report are for purposes such as scientific research education internal quality control within a company and internal product development within a company.

报告结束

*** The End of Report ***