

功效检测报告

EFFICACY TEST REPORT

报告编号 Report No: RG240337-3CE

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
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103B, 1st Floor, No. 88 Dong'an Road, Anzhen
Street, Xishan District, Wuxi City

样品名称 Sample name: 多聚磷酸钠（PolyP45）

申请项目 Test Item: 微循环/舒缓 Microcirculation/Soothing

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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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广州华测品鉴生物技术有限公司

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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	2024.09.06-2024.10.14		
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	微循环/舒缓 Microcirculation/Soothing	WI-GZB-744 化妆品舒缓、修红功效测试-体外微血管内皮细胞舒缓、修红指标表达含量测定* WI-GZB-744 Cosmetic Soothing and Red-Repairing Efficacy Test-In vitro Microvascular Endothelial Cell Soothing and Red-Repairing Index Expression Content Determination *	0.1%、0.01%、浓度样品对细胞中 VEGF 表达抑制率分别为 34.84%、39.01%。 The inhibition rates of VEGF expression in the cells were 34.84% and 39.01% at the concentrations of 0.1% and 0.01%, respectively	见附件：8.结果判定 See Attachment: 8. Result Determination	在本次实验条件下，0.1%、0.01%浓度样品的测试结果能够支持客户对于“微循环/舒缓”结论的宣称。 Under the conditions of this experiment, the test results of the 0.1% and 0.01% concentration samples can support the customer's claim of "microcirculation/soothing".
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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、细胞计数仪、多功能酶标仪、二氧化碳培养箱、低速离心机、离心机
Inverted microscope, cell counter, multifunctional microplate reader, carbon dioxide incubator,
low-speed centrifuge, centrifuge

2. 实验材料 Experimental materials

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，再筛选 0.1%、0.01%、0.001%三个浓度梯度进行后续功效测试实验。 The samples were diluted into five concentration gradients of 0.5%, 0.2%, 0.1%, 0.05% and 0.01% for cytotoxicity test, and then three concentration gradients of 0.1%, 0.01% and 0.001% were selected for subsequent efficacy test.
阳性对照 Positive controls	地塞米松 Dexamethasone.
细胞及代次 Cells and generations	微血管内皮细胞（hCMEC）购自欣润生物，毒性测试为 09 代，功效测试为 10 代。 The microvascular endothelial cells (hCMEC) were purchased from Xinrun Biology, the toxicity test was 09 generations, and the efficacy test was 10 generations.

3. 测试原理 Test principle

血管内皮生长因子 (VEGF) 是生长因子的亚家族，是重要的信号转导蛋白，参与血管发生和血管生成，当皮肤组织受损时，VEGF 能促进血管内皮细胞的增殖和肉芽组织新生毛细血管的形成。皮肤受损或过敏时，细胞的血管内皮生长因子(VEGF)高表达，从而导致毛细血管扩张，形成红血丝。因此，通过 LPS 诱导细胞损伤，高表达 VEGF，再通过测定给药后样品组的 VEGF 表达含量，评价样品对 VEGF 表达的抑制作用，从而评价样品是否具有舒缓、修红功效。

Vascular endothelial growth factor (VEGF) is a subfamily of growth factors and an important signal transduction protein involved in angiogenesis and angiogenesis. When skin tissue is damaged, VEGF can promote the proliferation of vascular endothelial cells and the formation of new capillaries in granulation tissue. When the skin is damaged or allergic, the vascular endothelial growth factor (VEGF) of cells is highly expressed, which leads to telangiectasia and the formation of red blood. Therefore, cell injury is

induced by LPS, VEGF is highly expressed, and the VEGF expression content of the sample group after administration is measured to evaluate the inhibitory effect of the sample on VEGF expression, so as to evaluate whether the sample has the effects of relieving and repairing red blood cells.

4. 实验方案 Experimental protocol

组别 Group	诱导条件 Induction conditions	培养条件 Cultivation 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	含不同浓度样品的完全培养基 Complete medium with different concentrations of 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 cytotoxicity

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. 细胞种板 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 100μL。接种结束后，放置于 CO₂ 培养箱中培养 24 h±2 h。

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 μL per well. After inoculation, the cells were

incubated in a CO₂ incubator for 24±2 hours.

5.4. 细胞加样 Cell loading

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, 空白对照孔中加入完全培养基, 每孔 200μL。将 96 孔板置于 CO₂ 培养箱中培养 24h±2 h。

The culture medium in the 96-well plate was discarded, and induction and administration operations were carried out. A culture medium contain a certain concentration of test substance and LPS is added into a test substance hole, a culture medium containing LPS is added into a model hole, a culture medium contain a positive control and LPS is added into a positive control hole, a complete culture medium is added into a blank control hole, and each hole is 200μL. The 96-well plates were incubated in a CO₂ incubator for 24±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 检测需根据人微血管内皮细胞生长因子 (VEGF) 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 of human microvascular endothelial growth factor (VEGF) assay kit.

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. VEGF 含量计算 VEGF content was calculated

6.2.1. VEGF 释放抑制率的计算公式 Formula for calculating the inhibition rate of VEGF release

VEGF 释放抑制率 (%) = (1 - T/C) * 100%

VEGF release inhibition rate (%) = (1-T/C) 100%

式中:

Where:

T—受试物 VEGF 相对含量平均值;

T — mean value of the relative content of VEGF in the test substance;

C—模型组 VEGF 相对含量平均值。

C — Mean value of relative content of VEGF in the model group.

6.2.2. VEGF 相对含量 (%) = T/C100% Relative content of VEGF (%) = T/C100%

式中:

Where:

T—受试物 VEGF 含量平均值;

T — mean value of VEGF content in the test substance;

C—模型组 VEGF 含量平均值。

C — Mean value of VEGF content 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. 微循环/舒缓 Microcirculation/Soothing

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, VEGF 含量显著性升高 ($P < 0.05$) 或模型组与空白对照相比, VEGF 含量上调 20%; 阳性对照与模型组相比, VEGF 含量显著性降低 ($P < 0.05$) 或阳性对照与模型组相比抑制率 ≥ 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 VEGF in the model group was required to be significantly higher than that in the blank control ($P < 0.05$), or the content of VEGF in the model group was increased by 20% compared with the blank control. The test system was considered to be effective when the VEGF content in the positive control was significantly lower than that in the model group ($P < 0.05$) or when the inhibition rate of the positive control was ≥ 20% compared with that of the model group.

统计酶标仪测得的各组平行孔间吸光度的标准差（Standard Deviation，SD），并计算平行孔变异系数（Coefficient of Variation，C.V），C.V 值≤20%，则认为试验平行性有效。

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

8. 结果判定 Result determination

样品的 VEGF 相对含量小于模型组，且与模型组相比具有显著性差异（P<0.05），则认为该浓度样品对 VEGF 表达具有抑制作用。

The relative content of VEGF in the sample was smaller than that of the model group, and there was a significant difference (P<0.05), so it was considered that the concentration of VEGF had an inhibitory effect on the expression of VEGF.

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。0.1%、0.05%、0.01%这三个浓度，与细胞共培养后细胞增殖率均大于等于 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。

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. 0.1%, 0.05% and 0.01%, and that cell proliferation rate aft being co-culture with the cells is greater than or equal to 85%, which indicates that the t 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(Mean±SD) (%) Cell activity (Mean ± SD) (%)	OD 值 C.V 值 (%) OD value C. V value (%)
BC	100.00±1.76	1.56
0.5%-样品 0.5%-Samples	2.93±2.71	16.94

0.2%-样品 0.2%-Samples	8.79±0.35	1.60
0.1%-样品 0.1%-Samples	91.05±4.58	4.39
0.05%-样品 0.05%-Samples	94.10±2.74	2.55
0.01%-样品 0.01%-Samples	98.73±3.46	3.10

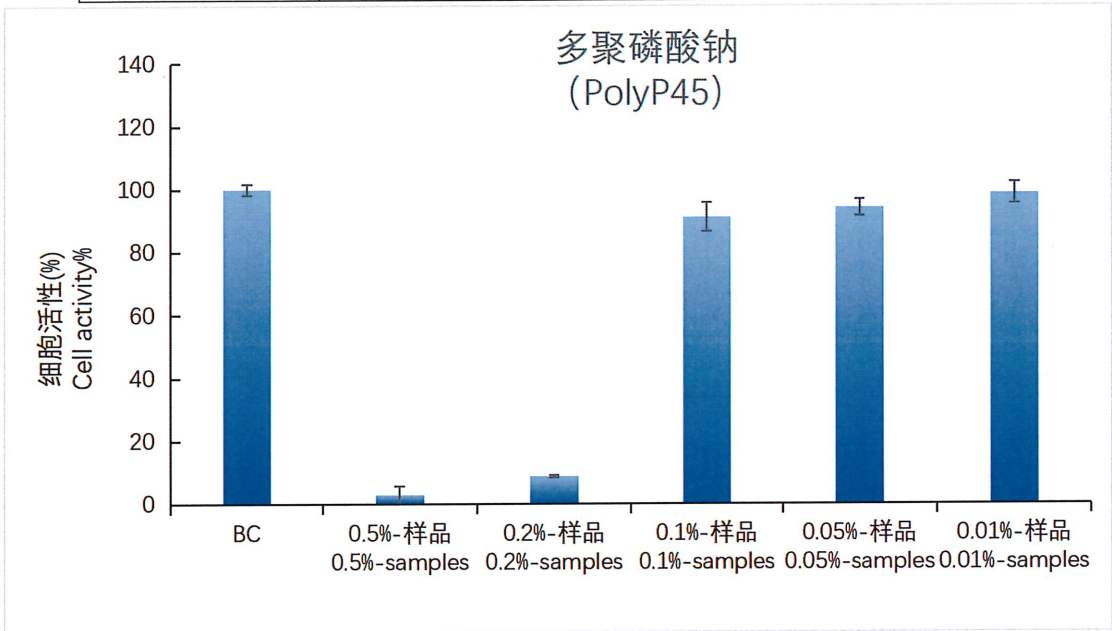


图 1.不同浓度样品的细胞活性 (Mean±SD)

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%三个浓度进行后续功效测试。

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

9.2. 体外 VEGF 含量测定结果 Determination of VEGF content in vitro

在本次测试条件下，模型组的 VEGF 相对含量为 100%，与 BC 组 VEGF 相对含量 42.53%相比显著升高，阳性对照组的 VEGF 相对含量为 53.15%，与模型组相比显著降低，表明试验系统有效。

Under this test condition, the relative content of VEGF in the model group was 100%, which was significantly higher than that in the BC group (42.53%), and the relative content of VEGF in the positive control group was 53.15%, which was significantly lower than that in the model group, indicating that the test system was effective.

样品在 0.1%、0.01%、0.001%测试浓度下处理细胞，其 VEGF 相对含量为 65.16%、60.99%、71.80%，

与模型组 VEGF 相对含量相比降低了 34.84%、39.01%、28.20%。具体数据见表 2、图 2 所示。

The relative content of VEGF was 65.16%, 60.99% and 71.80% when the cells were treated with 0.1%, 0.01% and 0.001% of the test concentration, which was 34.84%,39.01% and 28.20% lower than that of the model group. See Table 2 and Figure 2 for specific data.

表 2 不同组别细胞 VEGF 相对含量 (Mean±SD) 及抑制率

Table 2: Relative content (Mean±SD) and inhibition rate of VEGF in different groups

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

(*indicates that the difference was statistically significant compared to the model group, *P<0.05, **P<0.01)

测试组别 Test group	VEGF 相对含量 (Mean±SD) % Relative content of VEGF (Mean ± SD)%	VEGF 抑制率 (%) VEGF inhibition rate (%)
模型组 (M) Model Group (M)	100.00±1.34	N/A
空白组 (BC) Blank group (BC)	42.53±3.90**	57.47
阳性对照组 (PC) Positive control group (PC)	53.15±4.94**	46.85
0.1%-样品 0.1%-Samples	65.16±3.28**	34.84
0.01%-样品 0.01%-Samples	60.99±6.78**	39.01
0.001%-样品 0.001%-Samples	71.80±14.48	28.20

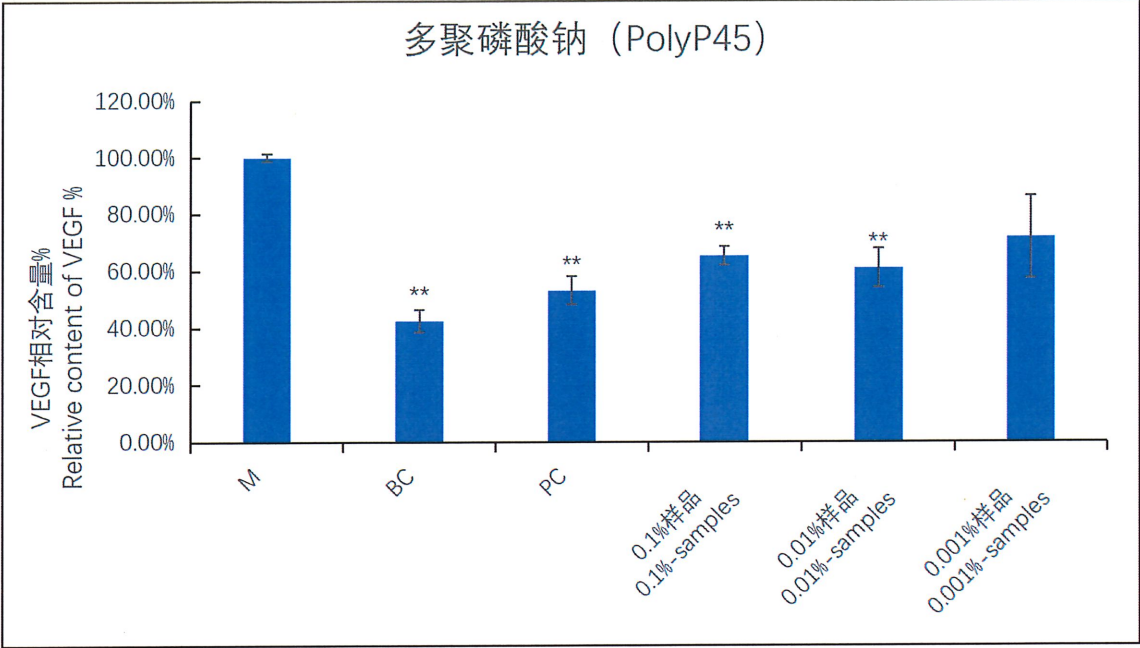


图 2 不同浓度样品处理后细胞中 VEGF 相对含量 (Mean±SD)

Fig. 2 Relative content of VEGF in cells treated with samples of different concentrations (Mean ± SD)

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

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

10. 结论 Conclusion

在本次实验条件下, 0.1%、0.01%浓度样品“多聚磷酸钠 (PolyP45)”与模型组相比, VEGF 相对含量显著降低, 0.1%、0.01%浓度样品的 VEGF 抑制率为 34.84%、39.01%, 说明 0.1%、0.01% 浓度的样品的测试结果能够支持客户对于“微循环/舒缓”结论的宣称。

In this experiment, the relative content of VEGF in the 0.1% and 0.01% concentration samples "PolyP45" was significantly reduced compared with the model group, and the VEGF inhibition rates of the 0.1% and 0.01% concentration samples were 34.84% and 39.01%, indicating that the test results of the 0.1% and 0.01% concentration samples can support the customer's claim of "microcirculation/soothing".

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 ***