

功效检测报告

EFFICACY TEST REPORT

报告编号 Report No: RG240337-7CE

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

样品名称 Sample name: 多聚磷酸钠 (PolyP45)

申请项目 Test Item: 改善牙龈健康 Improves gum health

报告发布日期 Date for Reporting: 2024.11.25

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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	2024.09.06-2024.10.17		
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	改善牙龈健康 Improves gum health	WI-GZB-727 化妆品抗皱、紧致功效测试-体外成纤维细胞抗皱紧致指标表达含量测定* WI-GZB-727 Cosmetic Anti-Wrinkle and Firming Efficacy Test-In Vitro Fibroblast Anti-Wrinkle and Firming Index Expression Content Determination*	样品在 0.1%、0.01%、0.001%测试浓度下与模型组相比，MMP1 基因相对表达量的抑制率分别为 32.63%、20.83%、25.78%。 Compare with a model group (M), that inhibition rate of the MMP1 gene relative expression quantity of the sample at the test concentration of 0.1%, 0.01% and 0.001% is respectively 32.63%, 20.83% and 25.78%.	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%、0.001%浓度样品的测试结果可以支持客户对于“改善牙龈健康”结论的宣称。 Under this experimental condition, the test results of 0.1%, 0.01% and 0.001% concentration samples can support the customer's claim of "Improves gum health" conclusion.
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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 Instruments

倒置显微镜、细胞计数仪、多功能酶标仪、二氧化碳培养箱、离心机、普通 PCR 仪、实时荧光定量 PCR 仪。

Inverted microscope,cell counter,multifunctional microplate reader,carbon dioxide incubator,centrifuge,common PCR instrument,real-time fluorescent quantitative PCR instrument.

2. 实验材料 Experimental materials

完全培养基 Complete medium	DMEM 高糖培养基+15%血清 High glucose DMEM + 15% serum
噻唑蓝（MTT）工作液 Thiazoleblue(MTT) working solution	用完全培养基稀释 5mg/mL MTT 储备液为 1mg/mL MTT 工作液，现配现用。 Dilute 5mg/ml MTT stock solution with complete culture medium to 1mg/ml MTT working solution, prepare and use immediately.
样品 Sample	取适量样品稀释成 0.5%、0.2%、0.1%、0.05%、0.01%五个浓度进行毒性实验；选择 0.1%、0.01%、0.001%三个浓度进行后续功效实验。 Appropriate amount of samples were diluted into five concentrations of 0.5%, 0.2%, 0.1%, 0.05% and 0.01% for toxicity test. Three concentrations of 0.1%, 0.01% and 0.001% were selected for subsequent efficacy experiments.
细胞及代次 Cells and generations	人皮肤成纤维细胞（HSF）购自丰晖生物，毒性测试为 15 代，功效测试为 10 代。（两次测试所用细胞为同一株细胞中的不同冻存管细胞） Human skin fibroblasts (HSF) were purchased from Fenghui Biology. The cell used for toxicity test was 15 generations and the toxicity test was 10 generations. (The cells used in two tests were cells in different cryopreserved tubes of the same cell strain.)

3. 实验原理 Experimental principles

基质金属蛋白酶（MMPs）是自然界进化中高度保守的一类酶，广泛分布于植物、脊椎动物、无脊椎动物中，是细胞外基质降解过程中必不可少的酶，几乎能降解细胞外基质的所有成分，包括胶原蛋白和弹性蛋白。如 MMP1 可水解细胞中 I、II、III 型胶原蛋白；MMP-3 参与水解 IV 型胶原蛋白、明胶、激活 MMP1/7/9 等。

Matrix metalloproteinases (MMPs) are highly conserved enzymes in nature, which are widely distributed in plants, vertebrates and invertebrates. They are essential enzymes in the process of extracellular matrix degradation, and can degrade almost all components of extracellular matrix, including collagen and elastin. For example, MMP1 can hydrolyze type I, II and III collagen in cells; MMP-3 is involved in the hydrolysis of type IV collagen, gelatin, activation of MMP1/7/9 and so on.

在正常稳定状态组织中 MMPs 表达量极少，而在炎症细胞因子、激素、生长因子等刺激下和细胞衰老过程中其表达量上升，因此可以通过 UVB 刺激诱导人成纤维细胞中 MMPs 表达上升，进行衰老细胞模型的建立，通过测定化妆品成品或原料给药后，衰老细胞中 MMPs 含量的下调情况，评价化妆品成品或原料在抑制 MMPs 表达方面是否具有功效。

The expression quantity of the MMPs is extremely low in a normal stable-state tissue, and the expression quantity of the MMPs is increased under the stimulation of inflammatory cytokines, hormones, growth factors and the like and in the cell aging process, so that the expression quantity of the MMPs in human fibroblasts can be induced to be increased through the UVB stimulation to establish an aging cell model, and after the administration of cosmetic finished products or raw materials, The down-regulation of MMPs content in senescent cells was used to evaluate the efficacy of cosmetic products or raw materials in inhibiting the expression of MMPs.

4. 实验方案 Experimental protocol

组别 Experimental 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	TBHP	完全培养基 Complete medium
阳性对照组 Positive control group	TBHP	含 TGF-β完全培养基 Complete medium containing TGF-beta
受试样品组 Test sample group	TBHP	含不同浓度样品的完全培养基 Complete medium with different concentrations of sampled

备注：诱导条件为客户指定。
Note: The inducement conditions are specified by the customer.

5. 实验步骤 Experimental steps

5.1. 细胞传代培养 Cell subculture

按照 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. 样品细胞毒性的测定 Determination of sample 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的样品浓度进行后续功

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

将对数生长期的细胞消化离心,以完全培养基配制成合适密度细胞悬液,按实验设计接种于 12 孔细胞培养板,于 37°C、5% CO₂ 培养箱内培养 24±2h。

Cells in logarithmic growth phase were digested and centrifuged to prepare cell suspension with appropriate density, which was inoculated into 96-well cell culture plate according to the experimental design, and cultured for 24 ± 2 hours at 37°C in 5% CO₂ incubator.

5.4. 细胞造模 Cell Modeling

除空白组外,模型组加入含有 TBHP 的完全培养基,阳性对照组加入含有 TBHP 和地塞米松的完全培养基,受试样品组加入含有不同浓度样品盒 TBHP 的完全培养基。

Apart from the blank group, the model group was supplemented with complete culture medium containing TBHP, the positive control group received complete culture medium containing both TBHP and dexamethasone, and the test sample groups were treated with complete culture media containing varying concentrations of the sample along with TBHP.

5.5. 细胞加样 dosing procedure

弃掉 12 孔板中的培养基,开展给药操作。样品组孔中加入含有不同浓度样品的完全培养基,阳性对照孔中加入含有阳性对照的完全培养基,空白对照孔和模型孔中加入完全培养基,每孔 1mL。加药完毕后将 12 孔板放置在 CO₂ 培养箱中培养 24±2h。

Discard the medium from the 12-well plate and perform the dosing procedure. Complete culture medium containing samples of different concentrations was added to the sample group wells, complete culture medium containing positive control was added to the positive control wells, and complete culture medium was added to the blank control wells and model wells, 1 mL per well. After dosing, the 12-well plate was placed in a CO₂ incubator for 24 ± 2H.

5.6. 样品收集 Sample collection

孵育培养结束后,弃掉细胞培养板中原培养液,用 DPBS 清洗一遍,进行细胞 RNA 提取实验。

After incubation and culture, the original culture medium of the cell culture plate was discarded and

washed once with DPBS, and the cell RNA extraction experiment was performed.

5.7. 细胞中基因表达量测定 Measurement of gene expression level in cell

将收集的细胞样本按照 WI-GZB-721 荧光定量法检测细胞中基因表达量操作规程进行 RNA 提取、cDNA 合成, 合成的 cDNA 产物按 1:1 比例用 DEPC 水进行稀释后再进行 qRT-PCR, 检测细胞中 MMPs 的表达量。

RNA was extracted and cDNA was synthesized from the collected cell samples according to the WI-GZB-721 fluorescence quantitative method for detecting the gene expression in the cells. The synthesized cDNA product was diluted with DEPC water at a ratio of 1:1 and then subjected to qRT-PCR to detect the expression of MMPs in the cells.

6. 数据分析 Data analysis

计量数据使用平均值±标准差 ($\bar{X} \pm s$) 来表示, 数据进行单因素方差分析, 用 P 值判断统计学差异, $P < 0.05$ 表示有统计学意义。

The measurement data were expressed by mean $\pm$ standard deviation ($\bar{X} \pm s$), and the data were analyzed by one-way ANOVA. The P value was used to judge the statistical difference, and $P < 0.05$ was statistically significant.

6.1. 细胞毒性的计算公式为: The calculation formula of cytotoxicity is:

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. 基因表达量计算方式为: The gene expression amount is calculated as follows:

使用 $2^{-\Delta\Delta CT}$ 方法对 qRT-PCR 试验得出的数据进行处理。处理得到的数据再进行统计学分析。

Data from the qRT-PCR assay were processed using the $2^{-\Delta\Delta CT}$ method. The data were processed and analyzed statistically.

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

7.1. 细胞毒性测试试验有效性判定: Determination of the effectiveness of the cytotoxicity test:

每组三个生物性重复的吸光度 OD 值变异系数 C.V 值 $\leq 20\%$ 。

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

7.2. 细胞中 MMPs 基因表达实验结果判定: The results of MMPs gene expression assay in cells were

determined:

每批次试验均须设置阳性对照, 要求每批次试验, 阳性对照均能检出 MMPs 基因相对表达量具有下调的效果, 且与模型组相比, 阳性对照 MMPs 基因相对表达量降低 0.2 倍, 则认为试验系统有效。

A positive control shall be set for each batch of tests, and the test system shall be considered to be effective if the positive control can detect that the relative expression of MMPs gene has a down-regulation effect, and the relative expression of MMPs gene in the positive control is 0.2 times lower than that in the model group.

qRT-PCR 实验要求每个样品浓度进行 3 次生物学重复, 且每个生物学重复进行 3 次技术性重复, 在每组的 3 个技术性重复值最多去掉 1 个误差较大值的条件下, 要求技术性重复间的标准差 (Standard Deviation, SD) ≤ 0.5 , 3 个生物学重复间 C.V 值 $< 20\%$, 则认为试验平行性有效。

The qRT-PCR experiment requires three biological replicates for each sample concentration, and three technical replicates for each biological replicate, and the Standard Deviation (SD) between technical replicates is required to be less than or equal to 0.5 under the condition that at most one larger error value is removed from the three technical replicate values of each group. If the C. V between the three biological replicates is less than 20%, the test parallelism is considered to be valid.

8. 结果判定 Result determination

样品 MMPs 的 mRNA 相对表达量小于模型组 (M), 且与模型组 (M) 相比具有显著性差异 ($P < 0.05$), 则认为该浓度样品具有抑制 MMPs 表达的作用。

The relative mRNA expression of MMPs in the sample was smaller than that of the model group (M), and there was a significant difference ($P < 0.05$) compared with the model group (M), so it was considered that the sample concentration had the effect of inhibiting the expression of MMPs.

9. 实验结果 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活性均 $\geq 85\%$, 表明 0.1% 及以下浓度样品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%, that cell activity after being co-culture with the cells is more than or equal to 85%, which indicates that the samples with the concentration of 0. 1% and below can be use for subsequent cell efficacy test experiments.

表 1 不同浓度样品组细胞活性（Mean±SD）

Table 1 Cell activity of different concentration sample groups (Mean ± SD)

组别 Group	细胞活性(Mean±SD)% Cell activity (Mean ± SD)%	OD 值 C.V 值(%) OD value C.V value (%)
BC	100.00±6.88	6.10%
0.5%-样品 0.5%-Sample	-0.40±0.55	4.49%
0.2%-样品 0.2%-Sample	17.70±1.20	3.96%
0.1%-样品 0.1%-Sample	97.01±9.14	8.34%
0.05%-样品 0.05%-Sample	97.12±5.50	5.01%
0.01%-样品 0.01%-sample	96.71±2.91	2.66%

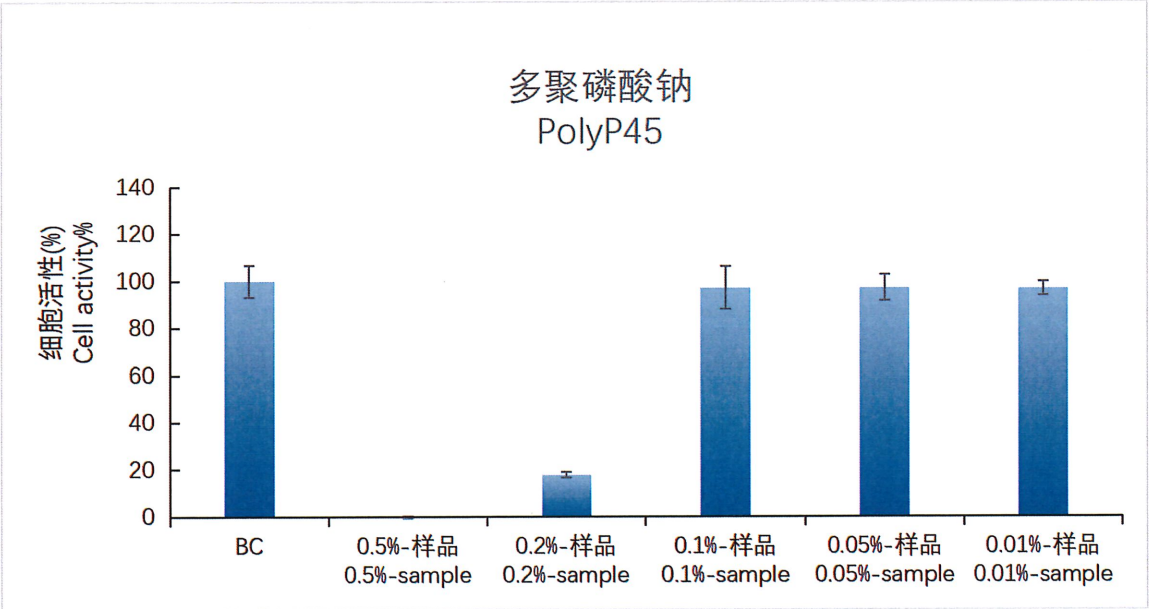


图 1. 不同浓度样品组的细胞活性（Mean±SD）

Fig. 1. Cell activity of different concentration sample groups (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。

Combined with 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. 样品对细胞中 MMP1 基因相对表达量的影响 Effect of sample on relative expression of MMP1

gene in cell

在本次实验条件下,空白对照组(BC)细胞中 MMP1 的基因相对表达量为 0.72,与模型组(M)相比降低了 27.61%,表明 UVB 照射处理后,模型组(M)细胞中 MMP1 的含量明显提高,衰老细胞模型造模成功,可以进行后续功效测试;

Under the conditions of this experiment, the relative expression of MMP1 gene in the cells of the blank control group (BC) was 0.72, which was decreased by 27.61% compared with the model group (M), indicating that after UVB irradiation, the content of MMP1 in the cells of the model group (M) was significantly increased, the aging cell model was successfully established, and the subsequent efficacy test could be carried out.

阳性对照组(PC)细胞中 MMP1 的基因相对表达量是 0.28,与模型组(M)相比降低了 72.26%,表明测试系统有效;

The relative expression of MMP1 gene in the positive control group (PC) was 0.28, which was 72.26% lower than that in the model group (M), 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 MMP1 基因相对表达量为 0.67、0.79、0.74,与模型组(M)相比抑制率分别为 32.63%、20.83%、25.78%,具体数据如表 2 和图 2 所示。

When the cells were treated with the samples at the test concentrations of 0.1%, 0.01% and 0.001%, the relative expression levels of MMP1 gene were 0.67, 0.79 and 0.74, and the inhibition rates were 32.63%, 20.83% and 25.78% respectively compared with the model group (M), and the specific data are shown in Table 2 and Figure 2.

表 2 不同浓度样品处理后细胞中 MMP1 的 mRNA 相对表达量及表达抑制率
Table 2 MMP1 mRNA relative expression and expression inhibition rate in cells treated with different concentrations of samples

(*表示与 M 对比差异具有统计学意义, *P<0.05 **P<0.01)

(* indicates statistically significant difference compared with M, * P < 0.05 ** P < 0.01)

实验组别 Experimental group	mRNA 相对表达量 (x±s) Relative mRNA expression (x ± s)	表达抑制率 (%) Expression inhibition rate (%)
M	1.00±0.05	N/A
BC	0.72±0.12*	27.61
PC	0.28±0.02**	72.26
0.1%-样品 0.1%-sample	0.67±0.08**	32.63

0.01%-样品 0.01%-sample	0.79±0.05*	20.83
0.001%-样品 0.001%-sample	0.74±0.09*	25.78

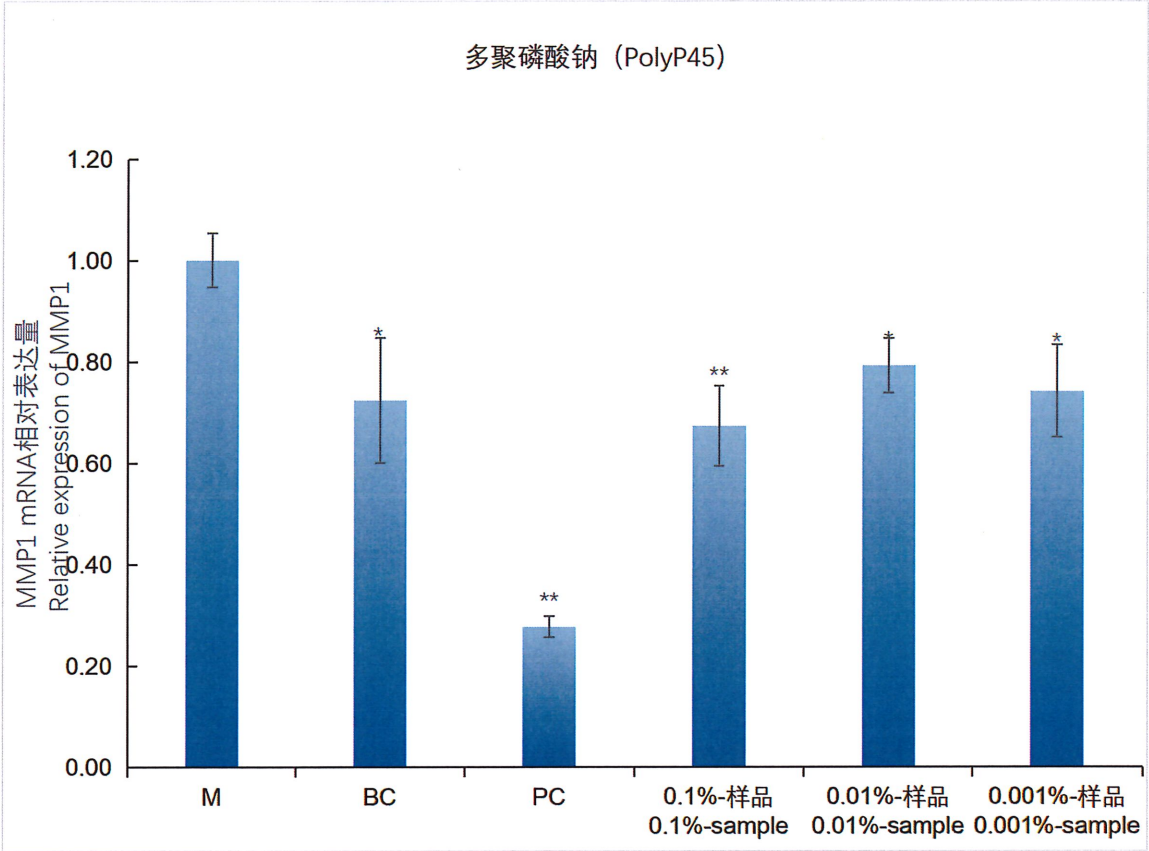


图 2. 不同浓度样品处理后细胞中 MMP1 的 mRNA 相对表达量 ($\bar{x} \pm s$)

Fig. 2. Relative expression of MMP1 mRNA in cells treated with different concentrations of samples ($\bar{X} \pm s$)

(*表示与 M 对比差异具有统计学意义, * $P < 0.05$ ** $P < 0.01$)

(* indicates statistically significant difference compared with M, * $P < 0.05$ ** $P < 0.01$)

10. 结论 Conclusion

在本次实验条件下, 样品“多聚磷酸钠 (PolyP45)”在 0.1%、0.01%、0.001%测试浓度下, 对细胞中 MMP1 基因表达具有显著抑制作用, 抑制率分别为 32.63%、20.83%、25.78%。0.1%、0.01%、0.001%浓度样品的测试结果可支持客户对于“改善牙龈健康”结论的宣称。

Under the conditions of this experiment, the sample "sodium polyphosphate (PolyP45)" had a significant inhibitory effect on MMP1 gene expression in cells at the test concentrations of 0.1%, 0.01% and 0.001%, and the inhibitory rates were 32.63%, 20.83% and 25.78% respectively.the test results of 0.1%, 0.01% and 0.001% concentration samples can support the customer's claim of "Improves gum health" conclusion.

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

* * * End of Report * * *