Report No.: BP-S-25013885-C3EN

# 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: | Skin sensitization test |
| Date of Issue: | 2025.05.13 |

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# First Page of Test Report

| | | | |
|---|---|---|---|
| Task No. | / | Detection category | Commission test |
| Sample No. | BP-S-25013885 | Sample source | Sent by client |
| Sample name | PLGA | Batch number | PLGA5050241127 10011100 |
| 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 2025.04.04 | | |
| Test item | Skin sensitization test | | |
| Test criterion | ISO10993-10:2021 | | |
| Test conclusion | Under the conditions of this experiment, the skin reaction grading of all animals in each period was grade 0, and the positive activation rate of the experimental group was 0%, indicating that the extract of the sample did not produce allergic reaction.<br>Date of issue: 2025.05.13 | | |
| Implementation standard | ISO/IEC 17025: 2017 | | |
| Remarks | "/" in the report indicates that this item is blank | | |

| Edited by | Checked by | Approved by (Authorized signatory) |
|---|---|---|
| 娄刚<br>Date: 2025.05.13 | 唐芳<br>Date: 2025.05.13 | Date: 2025.05.13 |

# Catalogue

## 1 Objective

The test was designed to evaluate the potential of a test article to cause skin sensitization using Guinea Pig Maximization Test.

## 2 Test method

Guinea Pig Maximization 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

| | | |
|---|---|---|
| Polar negative control sample：0.9%NaCl injection | | |
| Manufacturer | Shandong Qidu Pharmaceutical Co., Ltd. | |
| Specification | 500mL/bottle | |
| Batch No. | 15B24051403 | |
| Non-polar negative control: Cottonseed oil | | |
| Manufacturer | Shanghai Macklin Biochemical Technology Co., Ltd | |
| Specification | 13kg/drum | |
| Batch No. | C17477820 | |
| Positive control sample：1-Chloro-2,4-dinitrobenzene | | |
| Manufacturer | Shanghai Aladdin Biochemical Technology Co., LTD | |
| Specification | 100g/bottle | |
| Batch No. | D2420128 | |
| solvent | Absolute ethanol | |
| Concentration | Intradermal induction phase | 0.8% |
| | Topical induction phase | 0.8% |
| | Challenge phase | 0.2% |

## 4 Reagents and Instrument

### 4.1 Reagents

| Name | Supplier |
|---|---|
| 0.9%sodium chloride injection | Shandong Qidu Pharmaceutical Co., Ltd. |
| Cottonseed oil | Shanghai Macklin Biochemical Technology Co., Ltd |
| Complete Freund's Adjuvant | Sigma-Aldrich |
| SDS | Adamas-Beta® |

### 4.2 Instrument

| Name | Instrument ID | Calibration is valid until |
|---|---|---|
| Clean bench | WPE-TL0127 | 2025.10.09 |
| Shaking incubator | WPE-TL0081 | 2025.10.09 |
| Electronic balance | WPE-TL0029 | 2025.04.10 |
| pH meter | WPE-TL0394 | 2025.11.20 |
| Electronic counting scales | WPE-TL0055 | 2025.04.10 |

## 5 Test system

### 5.1 Test animal selection

The guinea pig is believed to be the most sensitive animal model for this type of study.

### 5.2 Test animal information

| | |
|---|---|
| Species | Albino guinea pig |
| Number | 30 |
| Sex | Male |
| Weight | 304g~396g |
| Age | Early adulthood |
| Health condition | Healthy |
| Adaptation | 5 days |
| Source | Jiashan Shengwang Ecological Farm, License number: SCXK（Zhe）2023-0010，Quality Certificate No. : 20250307Cdzz06000000055 |

5.3 Feeding and management

| | |
|---|---|
| Fodder | Wuhan Wanqian Jiaxing Biotechnology Co., Ltd., license No. : SCXK (E) 2021-0011 |
| Bedding | Wuhan Wanqian Jiaxing Biotechnology Co., Ltd., license No. : SCXK (E) 2021-0011 |
| Water | Free access to water |
| Cage | Suspended stainless steel cage |
| Environment | General environment, animal room 326, the temperature range of 18~29℃, humidity range of 30~70% |
| Light | Control cycle light (12 hours light, 12 hours dark) |
| Veterinary | Give necessary veterinary attention |
| Raise | The animals were raised in accordance with the "Feeding and management procedures of the experimental animal guinea pig" in our Toxicology laboratory |
| Certification body | The animal laboratory of this institution shall be certified by Shanghai Laboratory Animal Center, and the certifying authority: Shanghai Municipal Department of Science and Technology. Experimental animal use License No. : SYXK (Hu) 2021-0023 |
| Welfare | The IACUC established by the Institute confirmed that the experiment used a minimum number of animals without affecting the test results, and relevant documents were developed to safeguard animal welfare |

## 6 Experimental content

6.1 Sample preparation

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 | Stage | Actually sample | Sampling ratio | Solvent volume | Sampling condition | Whether it is clear | pH |
|---|---|---|---|---|---|---|---|
| 0.9%NaCl injection | Intradermal induction phase | 2.32g | 0.2g: 1mL | 15.31mL[a] | 37℃ 72h 60rpm | Yes | 6.17 |
| | Topical induction phase | 1.62g | | 10.69mL[a] | | Yes | 6.03 |
| | Challenge | 2.11g | | 13.92mL[a] | | Yes | 6.05 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | phase | | | | | | |
| Cottonseed oil | Intradermal induction phase | 2.16g | 0.2g: 1mL | 12.96mL[b] | 37℃ 72h 60rpm | Yes | 5.31 |
| | Topical induction phase | 1.53g | | 9.18mL[b] | | Yes | 5.45 |
| | Challenge phase | 2.12g | | 12.72mL[b] | | Yes | 5.45 |

a: Sample absorption rate is 1.60mL/g.

b: Sample absorption rate is 1.00mL/g.

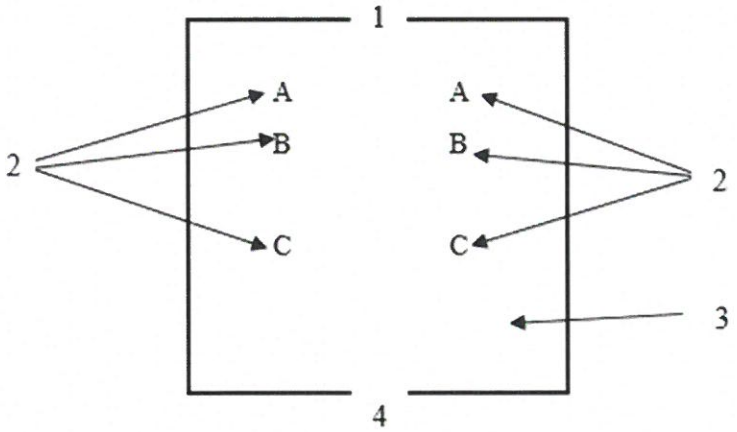
6.2 Test procedure

Before the experiment, the guinea pigs are marked and weighed. They are randomly divided into 0.9%NaCl injection test groups and control groups, Cottonseed oil test groups and control groups.

The skin reactions of guinea pigs are observed after 24h and 48h, and the body weight is recorded.

6.2.1 Intradermal induction phase

Make a pair of 0.1 mL intradermal injections of each of the following, into each animal, at the injection sites, as shown in Figure 1, in the clipped intrascapular region.




1——cranial end; 2——0.1mL intradermal injections; 3——clipped intrascapular region; 4——caudal end; A, B, C——injection sites

**Figure 1 — Location of intradermal injection sites**

Site A: A stable emulsifier mixed by injection of Freund's complete adjuvant with 0.9%NaCl injection / Cottonseed oil solvent in 50:50 (volume ratio) ratio.

Site B: The test sample (undiluted extract); inject the control animals with the extraction vehicle/solvent alone.

Site C: The test sample at the concentration used at site B, emulsified in a 50:50 volume ratio stable emulsion of Freund's complete adjuvant and the solvent/extraction vehicle; inject the control animals with an emulsion of the blank liquid with adjuvant.

There was no irritation at the test site, and the test area was pretreated with 10% sodium dodecyl sulfate 0.5mL (24±2) h before the application of the local dressing, and massaged into the skin.

6.2.2 Topical induction phase

At (7±1) d after the intradermal induction phase, according to the concentration selected in the intradermal induction site B, using an area of about 8cm$^2$ of absorbent gauze was used to saturate the test sample to cover the induced injection point, followed by a single layer. The bandages were covered with cellophane, fixed with non-stimulating tape, and removed after (48±2) hours Patch of application.

6.2.3 Challenge phase

At 14±1d after completion of the topical induction phase, challenge all test and control animals with the test sample. The local sticker is applied to the unbound site during the induction phase, and then covered with a layer of glass paper, and then fixed with no stimulating tape. After (24±2) h, remove the bandaging band and apply the fillet.

6.3 Observation

Observe the appearance of the challenge skin sites of the test and control animals (24±2) h and (48±2) h after removal of the dressings. Describe and grade the skin reactions for erythema and oedema according to the Magnusson and Kligman grading scale for each challenge site and at each time interval.

**Table 6-2 Magnusson and Kligman scale**

| Patch test reaction | Grading scale |
|---|---|
| No visible change | 0 |
| Discrete or patchy erythema | 1 |
| Moderate and confluent erythema | 2 |
| Intense erythema and/or swelling | 3 |

6.4 Evaluation standard

Magnusson and Kligman grades of 1 or greater in the test group generally indicate sensitization, provided grades of less than 1 are seen in control

animals. If grades of 1 or greater are noted in control animals, then the reactions of test animals which exceed the most severe reaction in control animals are presumed to be due to sensitization. If the response is equivocal, rechallenge is recommended to confirm the results from the first challenge. The outcome of the test is presented as the frequency of positive challenge results in test and control animals. Occasionally, the test group has a greater number of animals showing a response than the controls, although the intensity of the reaction is not greater than that exhibited by the controls. In these instances, a rechallenge can be necessary to define the response clearly. A rechallenge shall be carried out 1 week to 2 weeks after the first challenge. The method used shall be as described for the first challenge, using a naïve side on the animal.

## 7 Test result

The skin reaction grade and positive excitation rate of guinea pigs in this experiment are shown in Table 7-1, and the positive test results are shown in Table 7-2.

**Table 7-1 Result of skin sensitization reaction**

| Extraction solvent | Group | No. | Weight before test (g) | Weight after test (g) | Challenge phase grade | | positive activation rate(%) |
|---|---|---|---|---|---|---|---|
| | | | | | 24h | 48h | |
| 0.9%NaCl injection | Control | 1101 | 344 | 432 | 0 | 0 | 0 |
| | | 1102 | 345 | 454 | 0 | 0 | |
| | | 1103 | 345 | 458 | 0 | 0 | |
| | | 1104 | 355 | 447 | 0 | 0 | |
| | | 1105 | 337 | 452 | 0 | 0 | |
| | Sample | 2101 | 315 | 423 | 0 | 0 | 0 |
| | | 2102 | 318 | 428 | 0 | 0 | |
| | | 2103 | 331 | 428 | 0 | 0 | |
| | | 2104 | 341 | 448 | 0 | 0 | |
| | | 2105 | 328 | 427 | 0 | 0 | |
| | | 2106 | 336 | 429 | 0 | 0 | |
| | | 2107 | 351 | 456 | 0 | 0 | |
| | | 2108 | 338 | 442 | 0 | 0 | |
| | | 2109 | 366 | 470 | 0 | 0 | |
| | | 2110 | 384 | 486 | 0 | 0 | |
| Cottonseed oil | Control | 3101 | 396 | 500 | 0 | 0 | 0 |
| | | 3102 | 395 | 489 | 0 | 0 | |
| | | 3103 | 346 | 456 | 0 | 0 | |
| | | 3104 | 304 | 408 | 0 | 0 | |

| Extraction solvent | Group | No. | Weight before test (g) | Weight after test (g) | Challenge phase grade | | positive activation rate(%) |
|---|---|---|---|---|---|---|---|
| | | | | | 24h | 48h | |
| | | 3105 | 327 | 438 | 0 | 0 | |
| | Sample | 4101 | 336 | 443 | 0 | 0 | 0 |
| | | 4102 | 375 | 480 | 0 | 0 | |
| | | 4103 | 338 | 448 | 0 | 0 | |
| | | 4104 | 311 | 407 | 0 | 0 | |
| | | 4105 | 310 | 399 | 0 | 0 | |
| | | 4106 | 349 | 437 | 0 | 0 | |
| | | 4107 | 325 | 415 | 0 | 0 | |
| | | 4108 | 361 | 461 | 0 | 0 | |
| | | 4109 | 344 | 451 | 0 | 0 | |
| | | 4110 | 383 | 495 | 0 | 0 | |

**Table 7-2 Result of skin sensitization reaction in the positive control group**

| Group | No. | Weight before test (g) | Weight after test (g) | Challenge phase grade | | positive activation rate(%) |
|---|---|---|---|---|---|---|
| | | | | 24h | 48h | |
| Positive control | 2001 | 357 | 452 | 2 | 2 | 100 |
| | 2002 | 360 | 473 | 2 | 2 | |
| | 2003 | 329 | 448 | 1 | 2 | |
| | 2004 | 375 | 450 | 2 | 2 | |
| | 2005 | 346 | 444 | 2 | 2 | |
| | 2006 | 354 | 460 | 2 | 2 | |
| | 2007 | 363 | 452 | 1 | 2 | |
| | 2008 | 370 | 467 | 2 | 2 | |
| | 2009 | 346 | 458 | 2 | 2 | |
| | 2010 | 358 | 469 | 2 | 2 | |
| Remarks | Quote: report of project SHA-j-06-24120047-01-BC-01<br>Test date: 2024.12.05~2024.12.29 | | | | | |

## 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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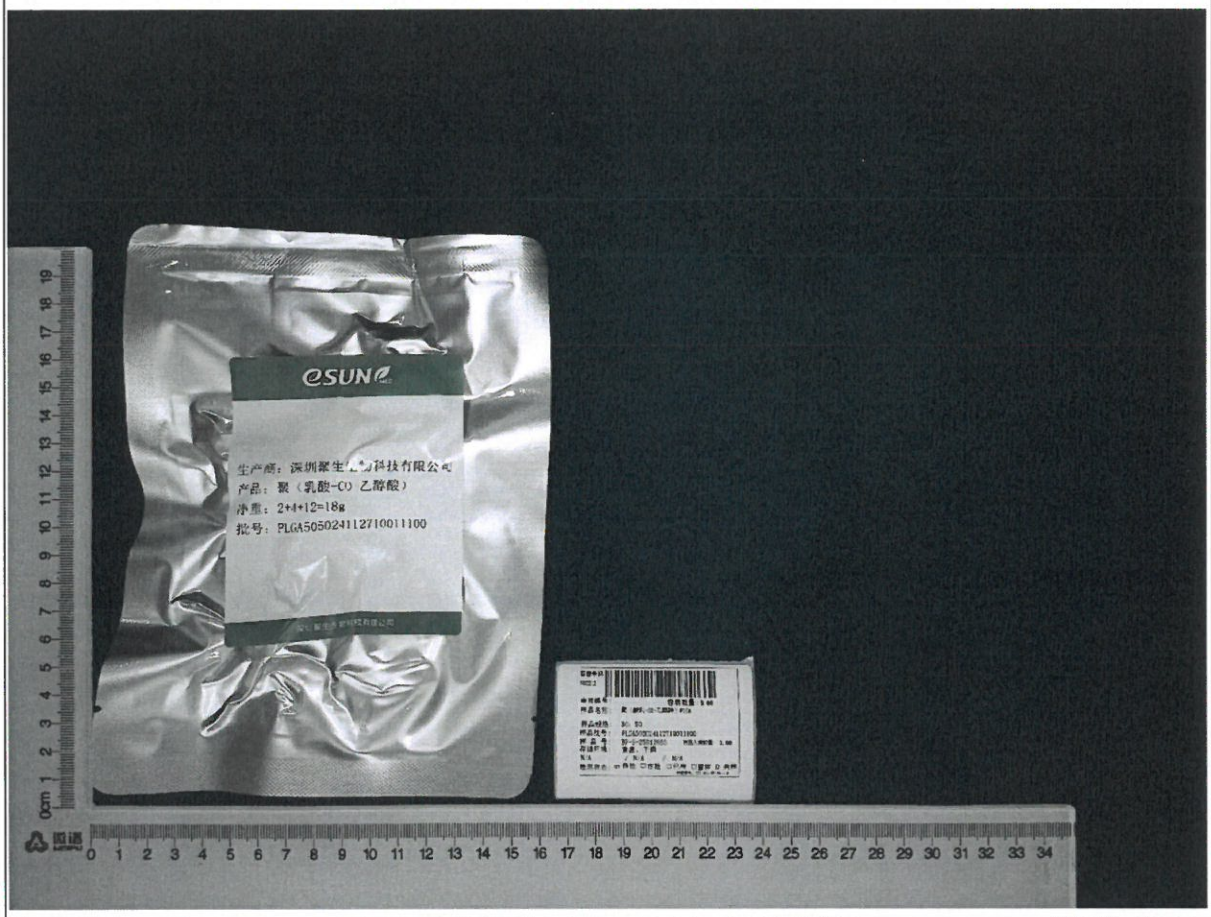
# Test report photo page

| Photos and descriptions |
|---|
|  |
| Test component description |
| Random sampling |
| Model, specification or other description |
| / |

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