Report No.: MZ6-191000001001EN

because results matter SUPER LAB

FINAL REPORT

Study title

Cytotoxicity Test

Test article name: Thermoplastic sheet

Sponsor: HYMED Technology Co.
No. 3-3, Hongmao, Xinfeng Township, Hsinchu County 30472, Taiwan

Test facility: Super Laboratory Co., Ltd. Contract Research Organization
No.21, Wugong 5th Rd., Xinzhuang Dist.,New Taipei City 24890, Taiwan (R.O.C.)

Remark:

- This report contains a total of 25 pages. It will be invalid if separated and/or partially copied.
- This test does not involve sampling of test article, and the final report is only applicable to the test article provided by sponsor.

SUPER LAB
because results matter

Study statement and signature:

This study was conducted in accordance with study protocol, and no test deviation or incident that would affect the integrity of this study. This study was conducted with test article information which provided by the sponsor. This study was conducted in compliance with Good Laboratory Practice for Nonclinical Laboratory Studies, FDA (21 CFR Part 58, 2018); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17, 1998) and Good Laboratory Practice for Nonclinical Laboratory Studies, Taiwan Food and Drug Administration (2019). (The sections of regulations excluded from this study: OECD GLP Section Ⅱ 6.1.3, 6.2.2, 6.2.4 and 6.2.5, FDA GLP Subpart F Sec.58.105 (a), (b), (c) and Sec. 58.113 (a), TFDA GLP Part Ⅱ 6.1.3, 6.2.2, 6.2.4 and 6.2.5).

Record and specimen reserve:

All raw data, record, study protocol and final report generated as a result of the study will be retained in archives of Super Laboratory Co., Ltd. The sample of test article was retained in "Test article room". The control period will be based on rule of the Super Laboratory Co., Ltd.

Signature of final report:

Study director: Yu-lun Chen 2019/12/19

Yu-lun Chen, Ph.D., Associate Research Fellow

Test facility management: Yueh-ting Tsai 2019/12/20

Yueh-ting Tsai, Ph.D., Vice President

Quality assurance statement:

The studies in this laboratory were conducted with study protocol (PR-MZ6-191000001EN) and SuperLab standard operating procedures, except the characteristics of the test article was provided by the sponsor. The Quality Assurance Unit (QAU) of Super Laboratory is responsible for auditing the study, raw data, and final report.

This study has been inspected by the QAU in accordance with standard operating procedure of Super Laboratory Co., Ltd. Results indicated no test deviation or incident that would affect the integrity of this study. The final report correctly described the methods and procedures used in the study, and accurately reflected the raw data generated during this study.

The QAU conducted inspections on the following dates. The findings were reported to the Study director and test facility management. The following list of inspected items, types and related dates:

Inspected item	Type of inspection	Inspected date	Reported date	
			Study director	Test facility management
Study protocol	study	2019/10/04	2019/10/04	2019/10/08
Raw data	study	2019/10/22	2019/10/22	2019/10/24
Final report	study	2019/10/22	2019/10/22	2019/10/24

Quality Assurance Unit: Ting-yu Li 2019/12/19

Ting-yu Li, Team Leader

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Cytotoxicity Test for "Thermoplastic sheet"

Abstract

This study was conducted according to ISO 10993-5: 2009 guideline to evaluate the cytotoxic effect of "Thermoplastic sheet" (Specimen ID: MZ6-191000001) supplied by HYMED Technology Co. Test included reagent control, negative control, positive control, and test group. All samples were extracted in Eagle's minimum essential medium (MEM) with 10% fetal bovine serum (FBS), then incubated with mouse fibroblast cell line (L929) for 24 hours (5 ± 1% CO_2, 37 ± 1°C). Morphology of cells (qualitative analysis) were examined by inverted microscope and cell viability was determined by MTT method (quantitative analysis). Results indicated that the cell of test group had no morphology changed or cytolysis, the qualitative morphological grading of cytotoxicity was classified as 0. Quantitative analysis showed that decrease of viability was 3.8% in test group. Therefore, the test article "Thermoplastic sheet" had no cytotoxic effects on mouse fibroblast cell line (L929) under the conditions designed for this study.

1. **Objective:**

This study was conducted according to ISO 10993-5: 2009 guideline to evaluate the cytotoxic effect of test article extracts in mouse fibroblast cell (L929).

2. **General information:**

2.1 Project No.: MZ6-191000001.

2.2 Sponsor information:

2.2.1 Sponsor title: HYMED Technology Co.

2.2.2 Sponsor address: No. 3-3, Hongmao, Xinfeng Township, Hsinchu County 30472, Taiwan.

2.2.3 Sponsor's representative: Hui-Yi Chiang.

2.3 Facility information:

2.3.1 Facility title: Super Laboratory Co., Ltd. Contract Research Organization.

2.3.2 Facility address: No.21, Wugong 5th Rd., Xinzhuang Dist., New Taipei City 24890, Taiwan (R.O.C.).

2.3.3 Study director: Yu-lun Chen, Associate research fellow.

2.3.4 Study person: Hsin-yi Lin, Analyst and Yu-ting Gu, Analyst.

2.3.5 Study director and person address: The same as facility address.

2.4 Testing sites:

Super Laboratory Co., Ltd. Contract Research Organization. 6F, No.21, Wugong 5th Rd., Xinzhuang Dist., New Taipei City 24890, Taiwan (R.O.C.).

3. **Study schedule:**

3.1 Study initiation date: 2019/10/04.

3.2 Experimental starting date: 2019/10/08.

3.3 Experimental completion date: 2019/10/09.

3.4 Study completion date: See study director's signature date in the final report.

4. **Test article (all information supplied by sponsor) and control article:**

4.1 Test article (Specimen ID: MZ6-191000001):

4.1.1 Article name: Thermoplastic sheet.

4.1.2 Receiving date: 2019/10/03.

4.1.3 Test article lot No., category, form, storage condition and expiration date were shown in Appendix 1 and 2.

4.2 Control article:

4.2.1 Negative control:

a. Article name: PE cling film.
b. Manufacturer: New Top Co.
c. Major ingredients: Polyethylene. Form: Film.
d. Storage condition: Room temperature.

4.2.2 Positive control:

a. Article name: Nitrile examination gloves.
b. Manufacturer: SHANGHAI MOTEX HEALTHCARE CO., LTD.
c. Major ingredients: Nitrile butadiene rubber. Form: Membrane.
d. Storage condition: Room temperature.

5. Test system and condition:

5.1 Cell line and incubation condition:

5.1.1 Cell line: Mouse fibroblast cell line (L929) (NCTC clone 929, BCRC RM60091) was purchased from the Food Industry Research and Development Institute, Taiwan.

5.1.2 Culture medium: Eagle's minimum essential medium (MEM) (including 2.0 mM L-Glutamine) with 10% fetal bovine serum (FBS).

5.1.3 Incubation condition: 37 ± 1°C incubator with 5 ± 1% CO_2.

6. Procedure:

6.1 Article extraction: This study was conducted according to ISO 10993-12: 2012 guideline and SuperLab standard operating procedures SOPP-301. Culture medium was served as the extraction vehicle. Negative control article, positive control article, and test article were extracted for 24 hours at 37 ± 1°C with constant agitation of 100 rpm. Culture medium without test article was placed in the same condition served as reagent control.

6.1.1 Reagent control: Culture medium.

6.1.2 Negative control: PE cling film was extracted in an extraction ratio of 6 cm^2/mL.

6.1.3 Positive control: Nitrile examination glove was extracted in an extraction ratio of 6 cm^2/mL.

6.1.4 Test article: The form of test article was sheet. It was extracted in an extraction ratio of 0.1 g/mL.

6.2 Qualitative analysis:

6.2.1 This study was conducted according to SuperLab standard operating procedure SOPP-107.

6.2.2 Cells were removed from culture flasks by enzymatic digestion (trypsin w/EDTA) and resuspended in culture medium. The cell suspension was adjusted at a density of 1.0 x 10^5 cells/mL.

6.2.3 Dispensed 1 mL of a cell suspension into each well of a 24-well plate (1.0×10^5 cells/well), and then incubated for 24 hours.

6.2.4 Removed the culture medium and replaced it with 1 mL extracts of culture medium, negative control, positive control, or the test article. All groups were tested in triplicate. The cells were incubated for 24 hours.

6.2.5 Cell morphology was graded and recorded according to the "Qualitative morphological grading of cytotoxicity of extracts" (ISO 10993-5: 2009) in Appendix 3.

6.3 MTT quantitative analysis:

6.3.1 This study was conducted according to SuperLab standard operating procedure SOPP-107.

6.3.2 Cells were removed from culture flasks by enzymatic digestion (trypsin w/EDTA) and resuspended in culture medium. The cell suspension was adjusted at a density of 1.0×10^5 cells/mL.

6.3.3 Dispensed 100 µL of a cell suspension into each well of a 96-well plate (1.0×10^4 cells/well), and then incubated for 24 hours.

6.3.4 Removed the culture medium and replaced it with 100 µL extracts of culture medium, negative control, positive control, or the test article. All groups were tested in triplicate. The cells were incubated for 24 hours.

6.3.5 Discarded the extracts and added 50 µL culture medium containing 1.0 mg/mL MTT. Wrapped the plate with aluminum foil to protect from light and incubated for 2 hours.

6.3.6 Discarded the medium containing MTT and added 200 µL DMSO, and then measured the absorbance 570 nm by an ELISA reader.

6.4 Statistical analysis:

6.4.1 Decrease of viability = [the sum of (the mean absorbance of the reagent control - the triplicated absorbance of reagent control, negative control, positive control or test group)] ÷ 3 ÷ the mean absorbance of the reagent control x 100%. If the value was less than 0.0%, then was showed as 0.0%.

6.4.2 The absorbance in quantitative analysis was shown as mean ± standard deviation (SD). The significant difference between groups were analyzed by SPSS software (Duncan's multiple range test of One-Way ANOVA). *p*-value < 0.05 was considered as statistically significant difference. If the decrease of viability was > 30% or the qualitative morphological grading of cytotoxicity was more than 2, that was classified as cytotoxicity.

6.5 Quality criteria:

6.5.1 The absorbance with 570 nm of reagent control group were greater than 0.2, and coefficient of variation was not more than 15%.

6.5.2 The decrease of viability of the positive control group was > 30% and the qualitative morphological grading of cytotoxicity was more than 2.

7. Results:

7.1 The qualitative analysis indicated that the cell morphology of reagent control, negative control and test groups after treating for 24 hours had no abnormality and cytolysis (Figure 1A, 1B and 1D). According to the "Qualitative morphological grading of cytotoxicity of extracts" (Appendix 3), the qualitative morphological grading of cytotoxicity of reagent control, negative control and test groups were 0. The cell morphology of positive control group showed significant cytolysis and disruption (Figure 1C), and the qualitative morphological grading of cytotoxicity was 4 (Table 1).

7.2 MTT quantitative analysis showed the absorbance with 570 nm of reagent control group, negative control group, positive control group and test group were 1.058 ± 0.059, 0.983 ± 0.057, 0.047 ± 0.001 and 1.018 ± 0.043, respectively. The decrease of viability in positive control group and test group were 95.6% and 3.8%, respectively (Table 2).

8. Discussion:

8.1 According to the evaluation criteria of ISO 10993-5: 2009 guideline, if the decrease of viability was > 30% or the qualitative morphological grading of cytotoxicity was more than 2, that was classified as cytotoxicity. Results of this study showed that the qualitative analysis of test article was level 0 of cytotoxicity. The decrease of viability in quantitative analysis was 3.8%. Thus, the test article had no cytotoxic potential on mouse fibroblast cell line (L929).

8.2 Quality check: The absorbance with 570 nm of reagent control group were greater than 0.2, and coefficient of variation was not more than 15%. The decrease of viability of the positive control group was > 30% and the qualitative morphological grading of cytotoxicity was more than 2. Thus, the credibility of study results was reliable.

8.3 The biocompatibility testing included cytotoxicity test, irritation test and sensitization test. For the overall assessment the biocompatibility characteristic of test article, it was recommended to consider the results with other biocompatibility reports.

9. Conclusion:

According to the evaluation criteria of ISO 10993-5: 2009 guideline, the qualitative analysis of test article was level 0 of cytotoxicity. The decrease of viability in quantitative analysis was 3.8%. Thus, the test article "Thermoplastic sheet" had no cytotoxic potential on mouse fibroblast cell line (L929) under the conditions designed for this study.

10. References:

10.1 Taiwan Food and Drug Administration (TFDA). Good Laboratory Practice for Nonclinical Laboratory Studies. 2019.

10.2 Food and Drug Administration (FDA). Good Laboratory Practice for Nonclinical Laboratory Studies. 21 CFR, Part 58. 2018.

10.3 International Organization for Standardization (ISO). Biological evaluation of medical devices-part 5: Test for *in vitro* cytotoxicity, ISO 10993-5, 2009.

10.4 International Organization for Standardization (ISO). Biological evaluation of medical devices-part 12: Sample preparation and reference materials, ISO 10993-12, 2012.

10.5 Organisation for Economic Co-operation and Development (OECD). OECD series on principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles of Good Laboratory Practice. ENV/MC/CHEM (98) 17. 1998.

Table 1: Qualitative morphological grading of cytotoxicity

Group [a]	Treatment time (hours)	Cell morphology	Qualitative morphological grading of cytotoxicity [b]
Reagent control	24	No cell lysis or morphology change	0
Negative control	24	No cell lysis or morphology change	0
Positive control	24	Nearly complete or complete destruction of the cell layers	4
Test	24	No cell lysis or morphology change	0

[a] Reagent control group: Extracts of culture medium; Negative control group: Extracts of PE cling film; Positive control group: Extracts of Nitrile examination gloves; Test group: Extracts of test article. All groups were tested in triplicate.

[b] Qualitative morphological grading of cytotoxicity was according to Appendix 3.

Table 2: Results of quantitative analysis by MTT method

Group [a]	Absorbance (570 nm) [b]	Decrease of viability (%) [c]
Reagent control	1.058 ± 0.059	0.0
Negative control	0.983 ± 0.057	7.1
Positive control	0.047 ± 0.001 *	95.6
Test	1.018 ± 0.043	3.8

[a] Reagent control group: Extracts of culture medium; Negative control group: Extracts of PE cling film; Positive control group: Extracts of Nitrile examination gloves; Test group: Extracts of test article.

[b] Each group was tested in triplicates, data were shown as mean ± SD.

[c] Decrease of viability = [the sum of (the mean absorbance of the reagent control - the triplicated absorbance of reagent control, negative control, positive control or test group)] ÷ 3 ÷ the mean absorbance of the reagent control x 100%. If the value was less than 0.0% showed as 0.0%.

* Represented significantly different from the reagent control ($p < 0.05$).

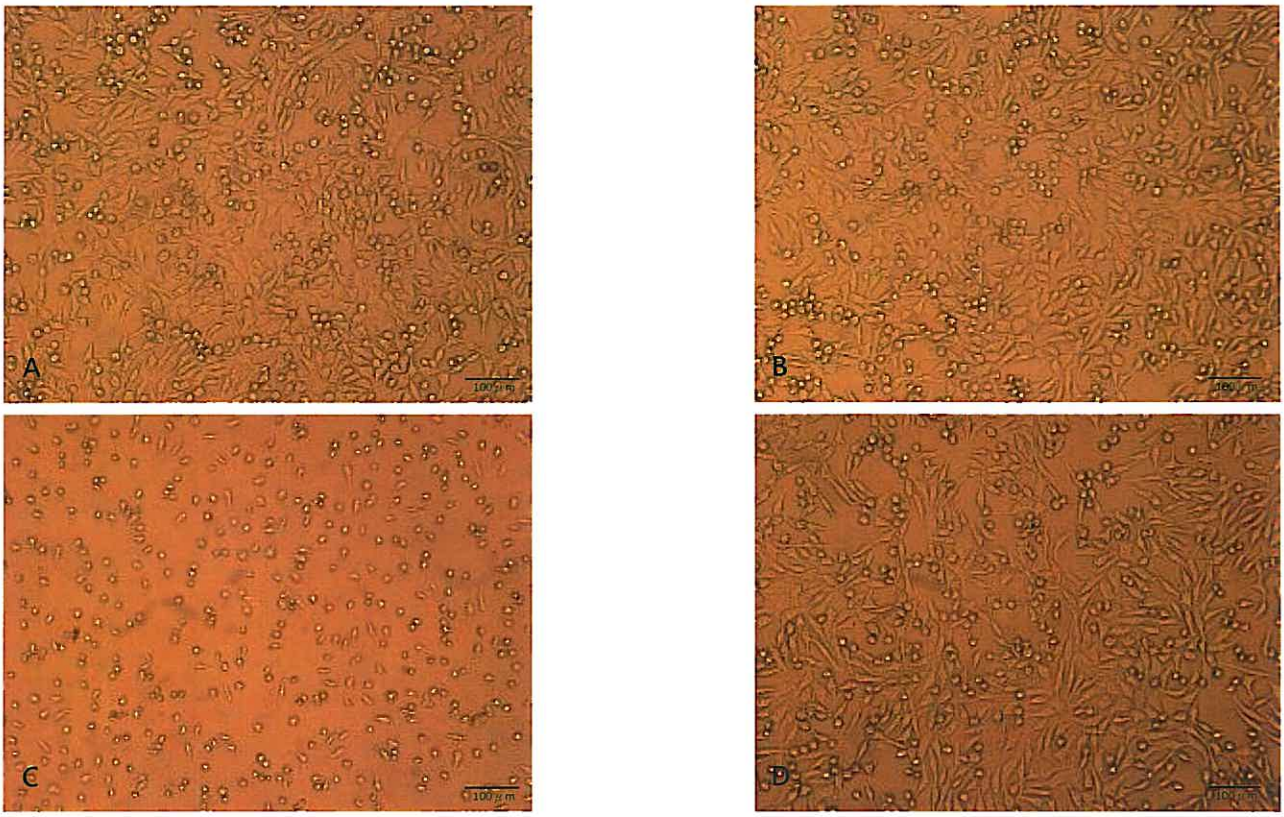

Figure 1: Cell morphology of mouse fibroblast cell line (L929) in all groups after 24 hours treatment. A: Reagent control group (extracts of culture medium); B: Negative control group (extracts of PE cling film); C: Positive control group (extracts of Nitrile examination gloves); D: Test group (extracts of test article). Cell morphology of reagent control, negative control and test groups had no abnormality and cytolysis. Positive control group showed significant cytolysis and disruption.

Appendix 1: The outer appearance of test article

Appendix 2: Test article information sheet (All data supplied by HYMED Technology Co.)

Test Article Information Sheet

Sponsor Information

Sponsor: HYMED Technology Co.	Sponsor's representative: Hui-Yi Chiang	
Sponsor's address: No. 3-3, Hongmao, Xinfeng Township, Hsinchu County 30472, Taiwan		
Sponsor's contact person: Kevin Yang	Tel: +886 910 784037	Fax:+886 3 5686961
Study item: Cytotoxicity Test、Rabbit Skin Irritation Test (Physiological Saline)、Rabbit Skin Irritation Test (Cottonseed Oil)、Guinea Pig Skin Sensitization Test (Maximization Method) (Physiological Saline)、Guinea Pig Skin Sensitization Test (Maximization Method) (Cottonseed Oil)		

Test Article Information

Article name: Thermoplastic sheet			
Lot No.:NA	Expiration date: NA (yyyy/mm/dd)		Color: Beige & White
Article category:	☐ Food ☐ Health food ☐ Drug ■ Medical devices ☐ Chemical ☐ Pesticides ☐ Other_____		
Article form:	☐ Liquid ☐ Powder ■ Sheet ☐ Granular ☐ Cream ☐ Capsular ☐ Membrane ☐ Other_____		
Packing specification/amount:	$25cm^2$/sheet × 15 sheets		(ex: 100 g/battle × 10 battles)
Major ingredients: Polyester & Polycaprolactone			
Sterilization:	■ No ☐ Yes, method: ☐ The article is aseptic and keep in aseptic condition		
Storage condition:	■ Room temperature ☐ Refrigeration (5 ± 3℃) ☐ Freezing (-20 ± 4 ℃) ☐ Protect from light ☐ Protect from moisture ☐ Other_____		
Disposal method for residual article:	■ Six months after the study was closed, discarded according to waste disposal standard operating procedures. ☐ Six months after the study was closed, discarded according to specified method by sponsor. Specified method: _____ ☐ Returned to sponsor		
Attachment	Stability test report	☐ Yes	■ No
	Certificate of analysis (COA)	☐ Yes	■ No
	Safety data sheet (SDS)	■ Yes	☐ No
	User's guide	☐ Yes	■ No
	Other:NA		
Precautions/Note:NA			

1. This is a required form which will be attached in final report. The unfilled field, please fill in NA.
2. "Sponsor's representative" must be the person who signs in study protocol.
3. This test article information sheet is an official document, please confirm the content was correct and sign.
4. The control date of retained article is five years from receiving date or based on the expiration date of test article.

Sponsor's representative sign and date (yyyy/mm/dd): Hui-Yi Chiang 2019/9/11

Appendix 3: Qualitative morphological grading of cytotoxicity of extracts

Grade	Reactivity	Conditions of all cultures [a]
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 completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

[a] According to ISO 10993-5: 2009 guideline.

Appendix 4: Study protocol

Protocol No.: PR-MZ6-191000001EN

STUDY PROTOCOL

Study title

Cytotoxicity Test

Test Article Name: Thermoplastic sheet

Sponsor: HYMED Technology Co.
No. 3-3, Hongmao, Xinfeng Township, Hsinchu County 30472, Taiwan

Test Facility: Super Laboratory Co., Ltd. Contract Research Organization
No.21, Wugong 5th Rd., Xinzhuang Dist., New Taipei City 24890, Taiwan (R.O.C.)

Protocol No.: PR-MZ6-191000001EN

Good laboratory practice statement:

This study will be conducted with test article information which provided by the sponsor. This study will be conducted in compliance with (1) Good Laboratory Practice for Nonclinical Laboratory Studies, Food and Drug Administration (FDA); (2) OECD Principles of Good Laboratory Practice, Organisation for Economic Co-operation and Development (OECD); (3) Good Laboratory Practice for Nonclinical Laboratory Studies, Taiwan Food and Drug Administration. (The sections of regulations excluded from this study: OECD GLP Section II 6.1.3, 6.2.2, 6.2.4 and 6.2.5, FDA GLP Subpart F Sec. 58.105 (a), (b), (c) and Sec. 58.113 (a), TFDA GLP Part II 6.1.3, 6.2.2, 6.2.4 and 6.2.5).

Record and specimen reserve:

All raw data, record, study protocol and final report generated as a result of the study will be retained in archives of Super Laboratory Co., Ltd. Expected archive file is shown in Appendix 1. The sample of test article will be retained in "Test article room". The control period will be based on rule of the Super Laboratory Co., Ltd.

Study approve:

Sponsor title/Representative: Hui-Yi Chiang 2019/10/04

HYMED Technology Co., Hui-Yi Chiang

Study director: Yu-lun Chen 2019/10/04

Yu-lun Chen, Ph.D., Associate Research Fellow

Protocol No.: PR-MZ6-191000001EN

Cytotoxicity Test for "Thermoplastic sheet"

1. Objective:

This study is conducted according to ISO 10993-5: 2009 guideline to evaluate the cytotoxic effect of test article extracts in mouse fibroblast cell (L929).

2. General information:
   - 2.1 Sponsor information:
     Sponsor title: HYMED Technology Co.
     Sponsor address: No. 3-3, Hongmao, Xinfeng Township, Hsinchu County 30472, Taiwan.
     Sponsor's representative: Hui-Yi Chiang.
   - 2.2 Facility information:
     Facility title: Super Laboratory Co., Ltd. Contract Research Organization.
     Facility address: No.21, Wugong 5th Rd., Xinzhuang Dist.,New Taipei City 24890, Taiwan (R.O.C.).
     Study director: Yu-lun Chen, Associate research fellow.
     Study director address: The same as facility address.
   - 2.3 Testing sites:
     Super Laboratory Co., Ltd. Contract Research Organization. 6F, No.21, Wugong 5th Rd., Xinzhuang Dist., New Taipei City 24890, Taiwan (R.O.C.).

3. Study schedule:
   - 3.1 Experimental starting date: 2019/10/08.
   - 3.2 Experimental completion date: 2019/10/09.

4. Test article (all information supplied by sponsor) and control article:
   - 4.1 Test article (Specimen ID: MZ6-191000001):
     - 4.1.1 Article name: Thermoplastic sheet.
     - 4.1.2 Receiving date: 2019/10/03.
     - 4.1.3 Source: HYMED Technology Co.
     - 4.1.4 Lot No.: NA.
     - 4.1.5 Storage condition: Room temperature. Expiration date: NA.

4.2 Control article:

4.2.1 Negative control:

a. Article name: PE cling film.
b. Manufacturer: New Top Co.
c. Major ingredients: Polyethylene. Form: Film.
d. Storage condition: Room temperature.

4.2.2 Positive control:

a. Article name: Nitrile examination gloves.
b. Manufacturer: SHANGHAI MOTEX HEALTHCARE CO., LTD.
c. Major ingredients: Nitrile butadiene rubber. Form: Membrane.
d. Storage condition: Room temperature.

5. Test system and condition:

5.1 Cell line and incubation condition:

5.1.1 Cell line: Mouse fibroblast cell (L929) (NCTC clone 929, BCRC RM60091) was purchased from the Food Industry Research and Development Institute, Taiwan.

5.1.2 Culture medium: Eagle's minimum essential medium (MEM) (including 2.0 mM L-Glutamine) with 10% fetal bovine serum (FBS).

5.1.3 Incubation condition: 37 ± 1°C incubator with 5 ± 1% CO_2.

6. Procedure:

6.1 Article extraction: This study will be conducted according to ISO 10993-12: 2012 guideline and SuperLab standard operating procedure SOPP-301. Culture medium is served as the extraction vehicle. Negative control article, positive control article and test article will be extracted for 24 ± 1 hours at 37 ± 1°C with constant agitation of 100 rpm. Culture medium without test article will be placed in the same condition served as reagent control.

6.1.1 Reagent control: Culture medium.

6.1.2 Negative control: PE cling film is extracted in an extraction ratio of 6 cm^2/mL.

6.1.3 Positive control: Nitrile examination glove is extracted in an extraction ratio of 6 cm^2/mL.

6.1.4 Test article: The form of test article is sheet. It is extracted in an extraction ratio of 0.1 g/mL.

6.2 Qualitative analysis:

6.2.1 This study will be conducted according to SuperLab standard operating procedure SOPP-107.

6.2.2 Cells are removed from culture flasks by enzymatic digestion (trypsin w/EDTA) and resuspended in culture medium. The cell suspension will be adjusted at a density of 1.0 × 10^5 cells/mL.

6.2.3 Dispense 1 mL of a cell suspension into each well of a 24-well plate (1.0×10^5 cells/well), and then incubate overnight.

6.2.4 Remove the culture medium and replace it with 1 mL extracts of culture medium, negative control, positive control, or the test article. All groups are tested in triplicate. The cells will be incubated for 24 ± 1 hours.

6.2.5 Cell morphology will be graded and recorded according to the "Qualitative morphological grading of cytotoxicity of extracts" (ISO 10993-5: 2009) in Table 1.

6.3 MTT quantitative analysis:

6.3.1 This study will be conducted according to SuperLab standard operating procedure SOPP-107.

6.3.2 Cells are removed from culture flasks by enzymatic digestion (trypsin w/EDTA) and resuspended in culture medium. The cell suspension is adjusted at a density of 1.0×10^5 cells/mL.

6.3.3 Dispense 100 µL of a cell suspension into each well of a 96-well plate (1.0×10^4 cells/well), and then incubate overnight.

6.3.4 Remove the culture medium and replace it with 100 µL extracts of culture medium, negative control, positive control, or the test article. All groups are tested in triplicate. The cells will be incubated for 24 ± 1 hours.

6.3.5 Discard the extracts and add 50 µL culture medium containing 1.0 mg/mL MTT. Wrap the plate with aluminum foil to protect from light and incubate for 2 ± 0.5 hours.

6.3.6 Discard the medium containing MTT and add 200 µL DMSO, and then measure the absorbance with 570 nm by an ELISA reader.

6.4 Statistical analysis:

6.4.1 Decrease of viability = [the sum of (the mean absorbance of the reagent control - the triplicated absorbance of reagent control, negative control, positive control or test group)] ÷ 3 ÷ the mean absorbance of the reagent control x 100%. If the value is less than 0.0%, then will be showed as 0.0%.

6.4.2 The absorbance in quantitative analysis will be shown as mean ± standard deviation (SD). The significant difference between groups will be analyzed by SPSS software (Duncan's multiple range test of One-Way ANOVA). p-value < 0.05 is considered as statistically significant difference. If the decrease of viability is > 30% or the qualitative morphological grading of cytotoxicity is more than 2, that is classified as cytotoxicity.

6.5 Quality criteria:

6.5.1 The absorbance with 570 nm of reagent control group are greater than 0.2, and coefficient of variation is not more than 15%.

6.5.2 The decrease of viability of the positive control group is > 30% and the qualitative morphological grading of cytotoxicity is more than 2.

7. Study report:

The final report should including but not be limited to the following item:

7.1 The characteristics of the test article (or based on information provided by sponsor).

7.2 Test system, control article and procedure.

7.3 The data generation and analysis methods of the result.

7.4 Result and conclusion of the study.

7.5 Copy of the study protocol.

8. References:

8.1 Taiwan Food and Drug Administration (TFDA). Good Laboratory Practice for Nonclinical Laboratory Studies. 2019.

8.2 Food and Drug Administration (FDA). Good Laboratory Practice for Nonclinical Laboratory Studies. 21 CFR, Part 58. 2018.

8.3 International Organization for Standardization (ISO). Biological evaluation of medical devices-part 5: Test for *in vitro* cytotoxicity, ISO 10993-5, 2009.

8.4 International Organization for Standardization (ISO). Biological evaluation of medical devices-part 12: Sample preparation and reference materials, ISO 10993-12, 2012.

8.5 Organisation for Economic Co-operation and Development (OECD). OECD series on principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles of Good Laboratory Practice. ENV/MC/CHEM (98) 17. 1998.

Protocol No.: PR-MZ6-191000001EN

Table 1: 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 completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

Appendix 1 : Expected archive file

Order	Name
1	Test service application form and attachment
2	GLP laboratory Study director/ Quality assurance unit assignment
3	Test article information Sheet
4	Safety data sheet (SDS)
5	Study protocol
6	Test article inspection form
7	Test article extraction recording sheet
8	Reagent preparation recording sheet
9	Attached cell subculture and cultivation standard operating procedure recording sheet
10	*In vitro* cytotoxicity test inspection procedure recording sheet
11	Data analysis
12	Final report

Appendix 5: Absorbance of 570 nm results

Group [a]	Absorbance (570 nm)		
	1	2	3
Reagent control	1.093	0.990	1.090
Negative control	1.018	1.014	0.917
Positive control	0.046	0.047	0.047
Test	1.054	0.970	1.030

[a] Reagent control group: Extracts of culture medium; Negative control group: Extracts of PE cling film; Positive control group: Extracts of Nitrile examination gloves; Test group: Extracts of test article.