[Form 5] Report on the Results of the Reverse Mutagenicity Test on Bacteria

1. General information

Name of new chemical substance (based on the IUPAC nomenclature system)			
Other name			
CAS no.			
Structural or rational formula (if neither is available, summarize its formulation method)			
Molecular weight			
Purity of the new chemical substance used for the test (%)			
Lot number of the new chemical substance used for the test			
Names and contents of impurities			
Vapor pressure			
Solubility in water			
1-Octanol/water partition coefficient			
Melting point			
Boiling point			
Properties at room temperature			
Stability			
Solubility in solvents, etc.	Solvent	Solubility	Safety in solvent

[Notes] Provide the physicochemical properties wherever possible.

1. Fill in the “Vapor pressure” column with the vapor pressure of the test substance.
2. Fill in the “Stability” column with the stability of the test substance against temperature, light, etc.
3. Fill in the “Solubility in solvents, etc.” column with the solubility and stability of the test substance in a solvent.

2. Strains of bacteria used in the test

Name of bacterial strain	Place it was obtained	Date it was obtained
		(day) (month) (year)
		(day) (month) (year)
		(day) (month) (year)
		(day) (month) (year)
		(day) (month) (year)

3. S9 mix

(1) Method, etc. of obtaining S9 (Circle the appropriate number and record the necessary information.)

Self-made or purchased	1. Self-made 2. Purchased (manufacturer)
Date of manufacture	Manufactured on (day) (month) (year)
If purchased, the Lot No.	
Storage temperature	℃

(2) S9 preparation method

Animals used		Inducing substances	
Type and species		Name	
Sex		Method of administration	
Age in weeks	Weeks	Administration period and administered dose (g/kg weight)	
Body weight	g		

(3) Composition of S9 mix

Ingredient	Amount in 1ml of S9 mix	Ingredient	Amount in 1ml of S9 mix
S9	ml	NADPH	μmol
$MgCl_2$	μmol	NADH	μmol
KCl	μmol	Na-Phosphoric acid buffering solution	μmol
Glucose-6-phosphoric acid	μmol	Other ( )	

4. Preparation of the test substance solution (Circle the appropriate numbers concerning the properties of the test substance solution and whether or not there is purity conversion.)

Solvent used	Name	Manufacturer	Lot No.	Grade	Purity (%)
Reason for selection of solvent					
Properties of the test substance solution	Dissolution Suspension Other ( )				
Methods for suspension, etc. when the test substance is very insoluble					
Solution storage time and temperature from the time of preparation to the time of use	hours minutes ℃				
Is there purity conversion?	Yes No				

5. Conditions, etc. for prior cultivation

(1) Conditions

Nutrient Broth	Name	Manufacturer	Lot No.
Time for prior cultivation	hours minutes		
Cultivation container (shape, volume)	ml		
Amount of culture solution	ml	Amount of inoculation bacteria	μl

(2) The number of viable bacteria, etc. at the time of completion of prior cultivation

Name of bacterial strain		Base pair substitution type			Frame shift type	
Number of viable bacteria ($x10^9$/ml)	Dose-range finding test					
	Main test					
Measurement method (Circle one.)		1. Conversion based on the O.D. value 2. Staged dilution method 3. Other ( )				

6. Minimal glucose agar plate culture medium (Circle the appropriate number and record the necessary information.)

Self-made or purchased	1. Self-made 2. Purchased (manufacturer)
Date of manufacture	Manufactured on (day) (month) (year)
If purchased, the Lot No.	
Name, manufacturer and Lot No. of the agar used	

7. Test method (Circle the appropriate number and record the necessary information)

(1) Test method and reason for selecting it

Adopted test method	1. Pre-incubation method 2. Plate method 3. Other ( )
If you chose "other," state your reason for selecting the alternative method	

(2) Test conditions

Composition	Bacterial suspension	ml
	Test substance solution	ml
	Na-phosphoric acid buffering solution (when using the direct method)	ml
	S9 mix (when using the metabolic activation method)	ml
	Top agar	ml
	Other ( )	
Pre-incubation	Temperature	℃
	Time	minutes
Incubation	Temperature	℃
	Time	hours

8. Colony measurement method

Measurement method	1. Manual measurement 2. Mechanical measurement
Are there any revisions?	1. No 2. Yes (Revision method:)

9. Test results

(1) Test results are in Appendix 1.

(2) Judgment of the results

Judgment (Circle one.) Positive Negative
Reason for judgment

(If judged positive, attach Appendix 2: Specific Activity.)

(3) Reference items

[Note] Record the views, etc. concerning the test results of the person responsible for the test in the “Reference items” field.

10. Others

Testing agency	Name	
	Address	Tel: Fax:
Test director	Name and status	
	Years of experience	
Test number		
Test period	From (month) (day) (year) to (month) (day) (year)	

[Notes]

1. Fill in the present form by transcribing from the final report.
2. Fill in the test number reported in the final report.
3. In the margin of this form, provide the name and affiliation of the person in charge of filling in this form.

(Appendix 1)

Test Results Table

Name of test substance: _____

Test period		From (day) (month) (year) to (day) (month) (year)					
Whether or not there is metabolic activation		Dosage of test substance (μg/plate)	No. of reverse mutations (No. of colonies/plate)				
			Base pair substitution type			Frame shift type	
-S9 mix		Negative control	( )	( )	( )	( )	( )
			( )	( )	( )	( )	( )
			( )	( )	( )	( )	( )
			( )	( )	( )	( )	( )
			( )	( )	( )	( )	( )
			( )	( )	( )	( )	( )
			( )	( )	( )	( )	( )
+S9 mix		Negative control	( )	( )	( )	( )	( )
			( )	( )	( )	( )	( )
			( )	( )	( )	( )	( )
			( )	( )	( )	( )	( )
			( )	( )	( )	( )	( )
			( )	( )	( )	( )	( )
			( )	( )	( )	( )	( )
Positive control	Positive control groups for which S9 mix is not necessary	Name					
		Dosage (μg/plate)					
		(No. of colonies/plate)	( )	( )	( )	( )	( )
	Positive control groups for which S9 mix is necessary	Name					
		Dosage (μg/plate)					
		(No. of colonies/plate)	( )	( )	( )	( )	( )

[Note]

1. If bacterial cell growth suppression is confirmed, mark the relevant figure with an asterisk in the top right corner.
2. Record the average number of colonies for each plate in the parentheses.
3. Record the measured values and average values for the number of reverse mutations in order of ascending test substance dosage.
4. If a sediment forms on the plate, mark that dosage with a †.
5. Record the names of positive substances shown in abbreviated form in the margin.

(Appendix 2)

Specific Activity

	Name of bacterial strain	–S9 mix		+S9 mix	
		Specific activity	Dosage used in calculation	Specific activity	Dosage used in calculation
Dose-range finding test					
Main test					