GENO-LAB
BACTERIAL GENOTOXICITY EDUCATIONAL KIT

STREAM

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Introduction

The Geno-Lab™ kit is a convenient approach for the detection and quantification of genotoxic activity in environmental air, water and sediment samples as well as pure chemicals, food components, cosmetics and biological fluids.

Purpose

The Geno-Lab™ kit utilizes native bacterial mechanisms for the detection of genotoxic damage in environmental samples. All living cells have developed a sensitive system for detection and repair of lesions to their genetic material that involve complex signalling pathways and enzymes. EBPI developed the Geno-Lab™ into a simple procedure, which can be performed in a non-specialized laboratory.

WARRANTY

EBPI warrants that, at the time of shipment, the Geno-Lab™ kit is free of defects in material and workmanship, and complies with the company specifications. Since actual experimental conditions prevailing at user's laboratory are beyond the control of EBPI or its representatives, EBPI makes no other warranty, express or implied, with respect to the product. Notification of any breach of warranty must be made within 120 days of delivery. The sole and exclusive remedy of the customer for any liability of EBPI of any kind, including liability based upon warranty (express or implied, whether contained herein or elsewhere) is limited to the replacement of the product or the refund of the invoice price of the product.

Concept: Testing for Genotoxicity

Genotoxic compounds are hazardous due to their ability to react negatively with cellular DNA and induce mutations or other physical damages that alter proper protein production and cellular function. These materials can induce genetic diseases like cancer which only emphasizes the importance of their detection in the environment.

The Geno-Lab™ kit utilizes native bacterial mechanisms for the detection of genotoxic damage in environmental samples. All living cells have developed a sensitive system for detection and repair of lesions to their genetic material that involve complex signaling pathways and enzymes. In bacteria, the SOS repair system is activated to repair damage of this kind. Once a DNA lesion has been detected, an SOS promoter is induced to start transcribing genes that code for repair proteins.

This system is sensitive and dependable which makes it an ideal target to exploit in the Geno-Lab™. Geno-Lab™ includes Geno-Express™ bacterial strains which have been engineered to express different human cytrochrome (CYP) P450 or glutathione-S-transferase GST T1-1 liver enzymes internally, promoting xenobiotic bioactivation and detoxification processes without S9 liver homogenate addition. The ability to create reactive metabolites through internal bioactivation improves assay response and preparation time by decreasing the proximity to DNA targets. Also, metabolite sequestration from S9 components like lipids and proteins does not occur.

Each Geno-Lab™ strain must be grown in media preconditioned with reagents to ensure proper enzyme expression. Each strain has an ideal growth value (OD) which will produce a significant positive test without excessive background rates of genotoxicity. If you are using the Geno-Express™ strains follow growth and exposure instructions for each individual strain given in Appendix 2 very carefully. Also, each bacterial strain has a positive control compound that produces a definitive response and verifies that the bacteria are behaving properly. After bacterial growth and dilution is complete for your individual strain, all other steps to run the assay are analogous to the traditional Geno-Lab™ and can be followed as stated in this booklet.

The Reaction

The bacteria used in the Geno-Lab™ is an engineered Escherichia Coli strain developed as an indicator organism for genotoxicity testing systems, and includes a gene coding for the β-galactosidase (β-gal) enzyme tethered to an SOS promoter. When DNA damage occurs, the SOS system is activated and β-gal gets transcribed proportionally to the level of SOS induction. Even limited repairable damage to genetic material is detected by the Geno-Lab™ due to the placement of the β-gal gene upstream of repair enzyme genes. Therefore, a positive response is produced regardless of cellular repair being initiated and the lesion being fixed.

The Geno-Lab™ bacterial strain has also undergone the following modifications to increase sensitivity to genotoxins:

- Endogenous DNA repair systems have been altered by a series of mutations so that even limited damage will not be repaired and will induce SOS response.
- The outer membrane of the cell has been modified to increase permeability to exogenous materials.
- The modified Geno-Lab™ promoter induces the synthesis of a readily detectable enzyme which produces a colour change when a chromogenic substrate is added. The amount of colour produced in the Geno-Lab™ is a quantitative measure of genotoxic damage to bacterial DNA.

Expectations

The Geno-Lab™ high school biotechnology kit is based on genetically engineered E.coli, which measures the primary response of a cell to genetic damage. This kit provides a clear, completely objective measurement of the genotoxicity of a sample. Once your class completes the experiment, the results can be recorded to determine genotoxicity levels and each group will complete a lab report for evaluation. The Geno-Lab™ is designed for students to work in six groups of three to four students.

Contents of the Geno-Lab™ Kit

Each kit contains sufficient components for one lab experiment. The bottles and vials in the Geno-Lab™ are labelled with clear, bold letters coded as follows:

	A: Growth medium for the Geno-Lab™ bacterial strain. (5 units)	**Geno-Lab™ Express Bacteria Reagents**	
	B: The Geno-Lab™ freeze-dried bacteria. (1 unit)		U: Reagent U (P450 1A2 and GST T1-1: 1 unit)
	C: 10% DMSO in saline; the Geno-Lab™ diluent. (1 unit)		W: Reagent W (P450 1A2 only: 1 unit)
	D: Standard genotoxic solution, containing 10 µg/mL 4 Nitroquinoline 1-oxide (4-NQO) in 10% DMSO saline. The 4-NQO has a Molecular Weight of 190.16. (1 unit)		X: Reagent X (P450 1A2 only: 1 unit)
	F: Blue chromogen solution. (2 units)		Y: Reagent Y (P450 1A2 only: 1 unit)
	J: Geno-Express overnight growth media (2 units)		Z: Reagent Z (P450 1A2 and GST T1-1: 1 unit)

DMSO: Pure DMSO solution, for dissolving water insoluble materials. (1 unit)	Required instrumentation (not included in the Geno-Lab™ kit): 1. Micropipettes with disposable tips in the range of 10 to 200 µL. 2. Spectrophotometer or photometer equipped with 600 (+/- 20) nm filter and using 1 cm light-path rectangular cuvettes (for preparation of the bacterial suspension). 3. A 37°C Incubator.

Collecting the Samples

You have the option of either having your students acquire the samples or you can acquire the samples. Four samples are required per group to conduct this lab. The samples should consist of chemical substances from home such as but not limited to pharmaceutical products (i.e. liquid based) and outdoor products (i.e. plant food, etc.). Ensure your students understand and follow safety measures when they are acquiring the samples. There should be a total of 24 samples organized as follows:

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
- Sample 1	- Sample 5	- Sample 9	- Sample 13	- Sample 17	- Sample 21
- Sample 2	- Sample 6	- Sample 10	- Sample 14	- Sample 18	- Sample 22
- Sample 3	- Sample 7	- Sample 11	- Sample 15	- Sample 19	- Sample 23
- Sample 4	- Sample 8	- Sample 12	- Sample 16	- Sample 20	- Sample 24

Student Station Preparation - Below is the suggested distribution of supplies for the preparation of the lab stations for the experiment:

FRONT OF THE CLASSROOM

For use by all Student Groups:
- OD_{600} Bacterial Suspension OR SOS Express Bacterial Strain
- Refrigerator (if available)
- Incubator (if available)

STUDENT WORKSTATION

For each Student Group:
- Microplate
- Geno-Lab diluent (50 uL)
- 4-NQO positive control (20 uL)
- Prepared sample dilutions (4 x 20 uL)
- Blue chromogen (6 mL)
- (?) volume of bacterial suspension in a clean vial or test tube (complete volume to 10 mL with fresh medium)

Preparation of the Samples

When acquiring and preparing the samples, it is important to ensure that the samples are biologically compatible with the Geno-Lab™. You also need to try to preserve the chemical integrity of the molecules within the samples so the genotoxic activity is representative of the sample itself and its origins. Please read the chart for further information on sample preparation:

10% DMSO in all Test Solutions	pH	Colour	Dilution of Samples	Complex Mixtures
Omitting or creating your own DMSO will increase the background signal and result in errors with your results and analysis. Only use the DMSO in our kit to all undiluted samples to a final concentration of 10% and dilute all samples in the kit.	The pH of the final sample preparation should be neutral (7.0 to 7.5) to ensure the reaction occurs. Be sure you check this and adjust accordingly before each test.	If the colour is faint, read the absorbance of the Geno-Lab plate IMMEDIATELY AFTER the addition of the chromogenic substrate mixture and remove this reading from the one taken at the end of the test after full blue colour development. If the colour is strong, a washing step must be included before the addition of the chromogenic substrate mixture. At the time of the reading of the plate, the addition of the chromogen must be taken and subtracted from the final value.	Whenever a new compound or mixture is tested, a wide range of dilutions will be required to ensure complete coverage of all possible concentrations. Therefore, test new samples serially diluted in two-fold dilutions over a broad range.	Most of the samples you will acquire from nature will be complex. The response of the Geno-Lab to a mixture is not predictable and may not be related to the sum of reactivities from the individual compounds present in the sample.

Evening before the Lab Experiment

1) Using aseptic techniques, open one bottle of COLD growth media (A) and one bottle of SOS bacteria (B). Immediately transfer the entire contents of the growth media from (A) to the dried bacteria in bottle (B).
2) Invert, mix well, and incubate at 37°C for 14 to 16 hours (overnight growth). By completing this process, the OD_{600} will reach an ideal value between .15-.20 when it is time to run the assay.

Note: Keep the remaining bottles of growth media (A) as it will be used for blanking the spectrophotometer and diluting bacterial suspension to the required OD of 0.05 at 600 (+/- 20) nm before use in the assay.

Day of the Lab Experiment

A) Start the preparation and dilution procedures as early as possible on the day of the lab experiment. Once these samples are properly diluted, they need to be properly dispensed into the appropriate wells of the 96 well microplate.

B) Before beginning this part, visually examine the bacterial suspension for turbidity indicating successful growth and continue ONLY if turbidity exists.

C) Remember to blank the machine using a fresh bottle of growth media (A). If rehydration and growth have been successful, the OD_{600} should be greater than 0.10.

If the average OD is between 0.1 and 0.2, the OD600 should be suspended to 0.05.	If the average OD is greater than 0.2, the OD600 should be suspended to 0.1.

Dissolving Your Sample and Preparing the Serial Dilutions

If you have time, your students can complete this part of the preparation as part of the lab experiment. Only one solvent should be used for all samples, controls, and blanks. This is provided in bottle (C) which consists of 10% dimethyl sulfoxide (DMSO) in sterile 0.85% saline. Do not use any other solvent.

Positive Controls	Solid Samples	Liquid Samples	Reaction Blanks
Bottle (D) contains a 10 ug/mL solution of 4-nitroquinoline-1-oxide (4-NQO) in a microfuge tube. This will be the first dilution to be used in the standard plot. Prepare six additional two-fold serial dilutions in 10% DMSO (C).	Dissolve your sample to be tested in water (or if insoluble in DMSO) in concentrations of 100-1,000 ug/mL or higher. Prepare at least 14 two-fold dilutions in 10% DMSO saline (C).	Prepare six two-fold dilutions in (C).	Use 10% DMSO saline (C).

SOS-Express™ Bacterial Strain Preparation - Please note that this needs to be done the day before the experiment.

Introduction: The correct combination of reagents is essential for proper bacterial growth. Overnight growth of SOS-Express™ bacteria takes place in SOS-Express overnight growth media **(J)** and should be carried out for at **LEAST 20 HOURS**. **After growth**, machine blanking and bacterial dilutions use SOS growth media **(A).** Ensure that the **SAME** reagents are added to the fresh growth media **(A)** used for bacterial dilution in the procedure.

To do in the evening prior to the experiment: Minimizing growth time produces bacteria in log growth phase which is ideal for chemical exposure. In the evening before the day of testing, using aseptic techniques, open one bottle of **COLD SOS-Express Overnight Growth Media (J)** and one bottle of SOS-Express Bacteria **(B)**.

Depending on the reagents you are using, please follow the procedure below for preparation:

SOS-Express P450 1A2 (EBP 128: Expressing CYP 450 1A2 and NAT enzymes)	**SOS-Express GST T1-1 (EBP 31: Expressing GST T1-1 enzyme)**
To prepare: Add 20 uL of Reagent U, 20 µL of Reagent W, 50 µL of Reagent X, 50 µL of Reagent Y, and 100 µL of Reagent Z to SOS-Express overnight growth media (J).	To prepare: Add 20 µL of Reagent U and 100 µL of Reagent Z to SOS-Express overnight growth media (J).
Open one vial of lyophilized bacteria and add the above nutrient mixture. Cover with rubber stopper, swirl and incubate.	Open one vial of lyophilized bacteria and add the above nutrient mixture. Cover with rubber stopper, swirl and incubate.

Immediately transfer approximately the entire contents of the growth media and reagents from **(J)** to the dried bacteria in bottle **(B)**. Invert, mix well and incubate at 37 ˚C for 20 to 22 hours.

By diluting the bacteria the night before, using cold growth media and starting bacterial growth late in the day, the OD_{600} will reach an ideal value between 0.20-0.30 when it is time to run the assay. The remaining bottles of SOS growth media **(A)** will be required for blanking the spectrophotometer and diluting bacterial suspension to the required OD of 0.10 at 600 (±20) nm before use in the assay (depending upon the degree of growth obtained).

Place bacterial suspension in 37°C incubator for overnight growth (20-22 hours).

Further Information for Educators

During the two hour incubation, genotoxic materials interact with the DNA of the Geno-Lab™ bacteria and induce the de novo synthesis of β galactosidase. At the last stage of the Geno-Lab™, the relative amount of enzyme produced as a result of the system induction, is measured by addition of a chromogenic substrate. Since the success of analysis and production of β galactosidase depend upon bacterial viability during the test, cell survival (ATP activity) is assessed using alkaline phosphatase. In this way, both acute bacterial toxicity as well as genotoxicity of the samples can be measured simultaneously.

Lab Safety Protocols – Review this with your class before beginning the experiment

Storage:

- The kit components should be stored under refrigeration (2°C to 8°C) or in a freezer (-4°C to -20°C) in total darkness if possible.
- If there is repeated freeze-thaw sequences and prolonged light exposure, the 4-NQO will degrade and affect the results.
- Light is a known DNA damaging agent, and the Geno-Lab™ bacterial strain is sensitive to it. Excess light will induce the colour reaction and increase background levels of induction. KEEP EXPOSURE TO LIGHT (especially shorter wavelengths) TO A MINIMUM.

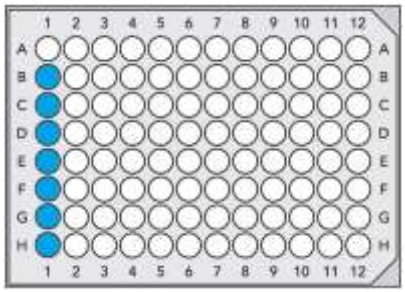
Handling:

- Handle the kit and tested samples like any potentially hazardous material.
- Collect all used components upon completing the assay and properly dispose according to laboratory protocols.
- Aseptic techniques should be employed when rehydrating the lyophilized bacteria. Cleanliness and aseptic techniques are strongly recommended for all laboratory procedures that involve bacteria.
- Note that the bacterial strain is not a known pathogen.

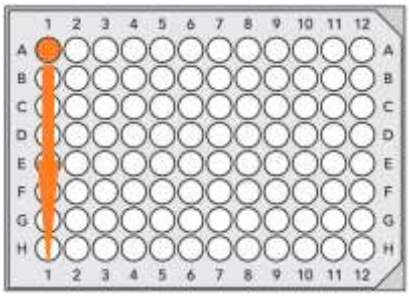
With the samples and preparation completed, you are now ready to begin the lab experiment. This part of the experiment is the most demanding. Make sure you know where each of the samples is to be dispensed.

Part 1: Dispensing the Samples and Controls into the Geno-Lab™ Microplate

1-1. Positive Control: Add 50 uL of Diluent (C) into wells (B-H) of column 1. Leave 1A empty.

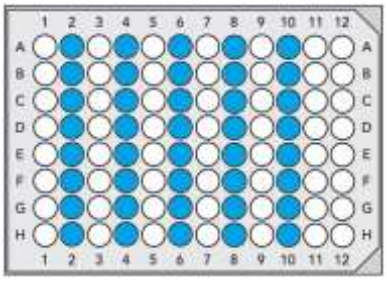


1-2. Dispense 20 uL of the 4-NQO control solution into 1A. Take 10 uL from this well and dispense into well 1B. Repeat this step down until you reach well 1H. Discard 10 uL from that well only.

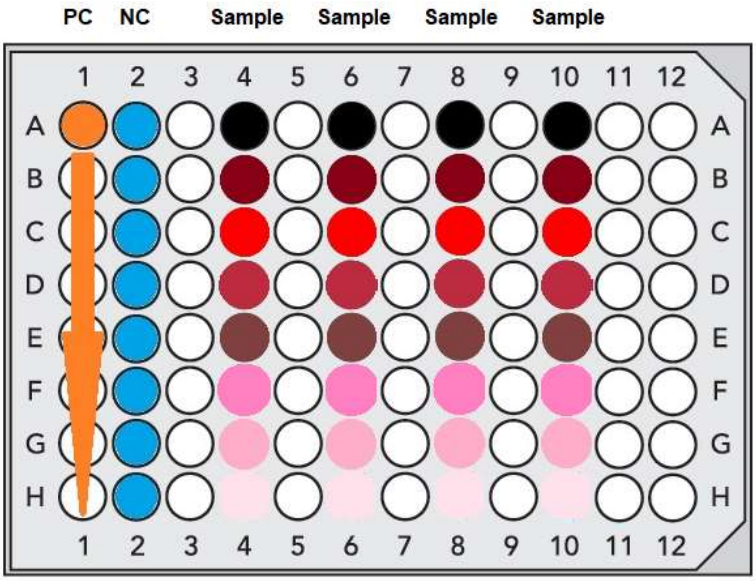


1-3. Add 50 uL of diluent (C) to all wells in rows A-H and columns 2, 4, 6, 8, and 10. Then add 10 uL of diluent (C) ONLY to wells 2A, 4A, 6A 8A, and 10A.

Column 2 receives only the diluent (C) and will act as the **negative control**.



1-4. Use Wells 4A, 6A, 8A, and 10A in columns 4, 6, 8, and 10 respectively to dispense 20 uL of your undiluted samples. Perform a series of dilutions by transferring 10 uL of sample down the column as you did with the positive control. Each sample should use two columns for sequential dilutions (i.e. use column 4 for one sample, column 6 for another sample, etc. Discard 10 uL from the final well in your dilution series.

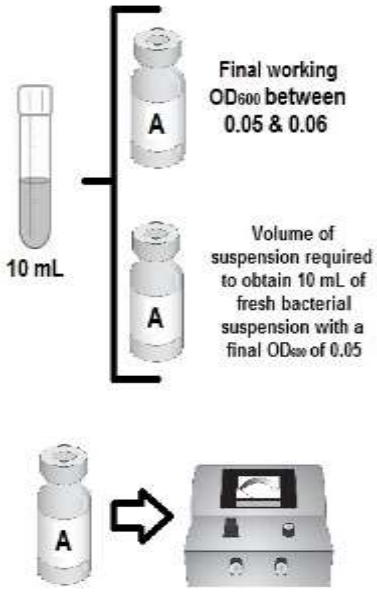


Part 2: Completing the Geno-Lab™

2-1. Using measured OD_{600} value for overnight suspension, dilute the bacteria in fresh growth media (A) to obtain a final working OD_{600} between 0.05 and 0.06.

If the overnight results show between 0.1 and 0.2 (OD_{600}), suspend to 0.05.

If the overnight results show greater than 0.2 (OD_{600}), suspend to 0.1.



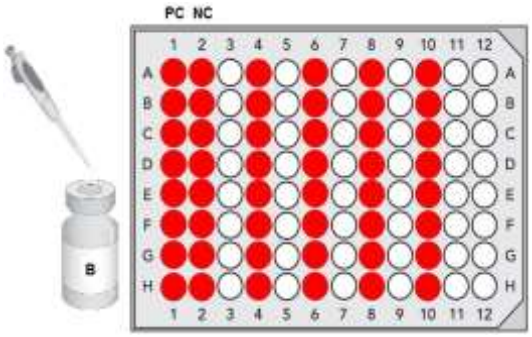
2-2. Take the overnight bacterial suspension and measure the OD at 600nm. Use a fresh bottle of growth media (A) to blank the spectrophotometer with a 1 cm light path cuvette.

Calculate the volume of suspension required to obtain 10mL of fresh bacterial suspension with a final OD600 of 0.05, using the following equation of the required volume (mL) of grown bacterial suspension to be added to the fresh growth medium to make 10 mL (VGM):

VGM = 0.5 / OD of growth suspension

2-3. Dispense the calculated 'Required Volume' of bacterial suspension (_____) into a clean vial or test tube. Complete the volume to 10 mL with fresh medium (A) (_____) and invert to mix.

Pipette 100 uL (0.1 mL) of freshly diluted bacterial suspension into each well of columns containing materials to be tested.



You will have **two options** to proceed from here:

Option A: Incubate the microplate for 2 hours at 37°C.

Time: _____

Once that is done, place the microplate in the refrigerator overnight. The next day you can add the chromogen (100 uL).

OR

Option B: Leave the microplate overnight at room temperature (approximately 20°C). The next day you can add the chromogen (100 uL).

Please note the following:

If a light blue colour fails to develop in the wells containing the test material this likely means that the material being tested was acutely toxic.

If a light blue colour fails to develop in the wells containing reagent blank with no test material this likely means that some other procedural issue resulted in the death of the bacteria.

To Educators: Please review the information as a visual guideline of the experiment results, recording the results, further analysis, and the answer sheet for the questions.

Observing the Results

The Geno-Lab™ will help with the analysis of the results and produce definitive colour changes to check bacterial function according to the procedure given. Students will be able to visually analyze the genotoxic activity of a tested material.

Criteria for a Valid Test: For the Geno-Lab™ assay to be considered valid, the following conditions must be met:	a) **Positive Control (Column 1)**: The wells in column 1 should contain a series of 2-fold dilutions of the positive control (4-NQO). A blue colour should appear with the darkest blues appearing at the top of the column with different densities corresponding to the compound concentration. There should be no blue colour development or concentration gradient in the positive control column (column 1). If this occurs, the results obtained are invalid. b) **Negative Control (Column 2)**: The wells in column 2 are used as negative control wells containing diluent and bacteria but NO samples. Small amounts of colour change will be produced in accordance with background levels of enzyme production in the absence of induction. If there is no colour production in these wells, the bacteria are not viable and have died.

Sample Results of a Geno-Lab™:

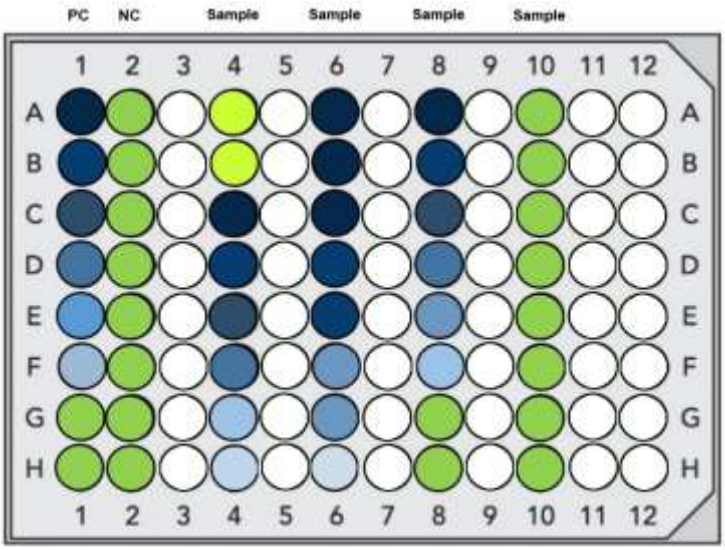


Figure 1: Sample Geno-Lab™ Results

Components
- Column 1: Positive control (4-NQO)
- Column 2: Negative control that contain Geno-Lab™ diluent and bacteria only
- Columns 4, 6, 8, and 10: Unknown samples diluted in each well down the column

Qualitative Results Guidelines:
- Sample 1 in column 4 is genotoxic at lower concentrations (blue wells) and cytotoxic at the highest concentrations (yellow wells).
- Sample 2 in column 6 is evidently genotoxic and seem to demonstrate a dose response relationship (blue wells, gradually getting lighter).
- Sample 3 in column 8 is also evidently genotoxic and seem to demonstrate a dose response relationship (blue wells, gradually getting lighter).
- Sample 4 in column 10 is not genotoxic at all (green wells correspond to background expression of alkaline phosphatase and β-galactosidase).

Recording the Results

1. Students will first record their own group observations in the 'Geno-Lab™ Results Table.' Record the blue colour intensity of the tested material giving reactions and assign values to them (see below for a comparative chart). Compare with values obtained for the 4-NQO for reference (using a digital photo) to other materials you may have tested or will test in the future.

The following is a checklist for students to follow when recording results. Use the sample results (Figure 1 – previous page) as a visual guideline:

1) Check the positive 4-NQO control (column 1).
2) Check the **blue** colour density appearing in the wells of your test materials and continuously compare with the diluent-only wells.
3) Start checking from the highest concentration of the test material to the lowest.
4) High concentrations may not induce any positive response due to acutely toxic concentrations in which the cells are killed outright.
5) As the material is diluted out, toxicity is reduced and a positive reaction (**deep blue** colour) may then appear indicating chronic genotoxicity.

The colour density will fade into the background **yellow-blue** as diluted concentrations fall below genotoxic levels.

When you analyze your results, please note below:

Visual Results

The degree of genotoxicity levels will be classified into five categories based on a visual inspection of the plate wells of your samples that were tested:

(-)	Yellow-blue colour present and no genotoxicity present
(+/-)	Light blue & yellow-blue colouring indicating low genotoxicity levels
(+)	< 50% blue colour intensity compared to the control, moderate genotoxicity levels
(++)	< 100% but > 50% colour intensity compared to the control, high genotoxicity levels
(+++)	Blue colour that is equivalent to positive control and very high genotoxicity levels

Comparisons should be made to the average blue development from the positive control column (Column 1). See the left side.

Please read below as well to assist with the recording of the results:

No positive blue colour: Check the yellow colour density appearing in the wells of your test materials, following the addition of alkaline phosphatase and subsequent incubation.	**The presence of Yellow colour**: If the yellow colour appearing in the wells of the test material is similar to the background (diluent-only wells), the material was not toxic and not genotoxic.	**Yellow in the diluent control**: Try higher dilutions (lower concentrations) of test material so that inherent acute toxicity will not be expressed.

2. Once this occurs, have each group will go to the other group results and record those results within the 'Geno-Lab™ Results Table.' Have the students compare the amount of colour development in each sample dilution. Please note that the colour development between the samples will vary depending on the type of samples used. If there are some samples that are relatively similar, these results can be grouped together.

Sample Geno-Lab™ Results Table Template

Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)
1	1 (A)		2	1 (A)		3	1 (A)	
	2 (B)			2 (B)			2 (B)	
	3 (C)			3 (C)			3 (C)	
	4 (D)			4 (D)			4 (D)	
	5 (E)			5 (E)			5 (E)	
	6 (F)			6 (F)			6 (F)	
	7 (G)			7 (G)			7 (G)	
	8 (H)			8 (H)			8 (H)	

Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)
4	1 (A)		5	1 (A)		6	1 (A)	
	2 (B)			2 (B)			2 (B)	
	3 (C)			3 (C)			3 (C)	
	4 (D)			4 (D)			4 (D)	
	5 (E)			5 (E)			5 (E)	
	6 (F)			6 (F)			6 (F)	
	7 (G)			7 (G)			7 (G)	
	8 (H)			8 (H)			8 (H)	

Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)
7	1 (A)		8	1 (A)		9	1 (A)	
	2 (B)			2 (B)			2 (B)	
	3 (C)			3 (C)			3 (C)	
	4 (D)			4 (D)			4 (D)	
	5 (E)			5 (E)			5 (E)	
	6 (F)			6 (F)			6 (F)	
	7 (G)			7 (G)			7 (G)	
	8 (H)			8 (H)			8 (H)	

Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)
10	1 (A)		11	1 (A)		12	1 (A)	
	2 (B)			2 (B)			2 (B)	
	3 (C)			3 (C)			3 (C)	
	4 (D)			4 (D)			4 (D)	
	5 (E)			5 (E)			5 (E)	
	6 (F)			6 (F)			6 (F)	
	7 (G)			7 (G)			7 (G)	
	8 (H)			8 (H)			8 (H)	

Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)
13	1 (A)		14	1 (A)		15	1 (A)	
	2 (B)			2 (B)			2 (B)	
	3 (C)			3 (C)			3 (C)	
	4 (D)			4 (D)			4 (D)	
	5 (E)			5 (E)			5 (E)	
	6 (F)			6 (F)			6 (F)	
	7 (G)			7 (G)			7 (G)	
	8 (H)			8 (H)			8 (H)	

Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)
16	1 (A)		17	1 (A)		18	1 (A)	
	2 (B)			2 (B)			2 (B)	
	3 (C)			3 (C)			3 (C)	
	4 (D)			4 (D)			4 (D)	
	5 (E)			5 (E)			5 (E)	
	6 (F)			6 (F)			6 (F)	
	7 (G)			7 (G)			7 (G)	
	8 (H)			8 (H)			8 (H)	

Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)
19	1 (A)		20	1 (A)		21	1 (A)	
	2 (B)			2 (B)			2 (B)	
	3 (C)			3 (C)			3 (C)	
	4 (D)			4 (D)			4 (D)	
	5 (E)			5 (E)			5 (E)	
	6 (F)			6 (F)			6 (F)	
	7 (G)			7 (G)			7 (G)	
	8 (H)			8 (H)			8 (H)	

Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)
22	1 (A)		23	1 (A)		24	1 (A)	
	2 (B)			2 (B)			2 (B)	
	3 (C)			3 (C)			3 (C)	
	4 (D)			4 (D)			4 (D)	
	5 (E)			5 (E)			5 (E)	
	6 (F)			6 (F)			6 (F)	
	7 (G)			7 (G)			7 (G)	
	8 (H)			8 (H)			8 (H)	

Further Analysis

You can pick further analysis questions based on your preference and the project stream's overall objectives.

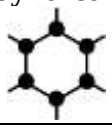
Questions and Answers

1. Explain how genotoxicity can be harmful to our bodies.	Genotoxic can cause harmful mutations in cellular DNA or other physical damages that alter proper protein production and cellular function. These materials can induce genetic diseases like cancer which only emphasizes the importance of their detection in the environment.
2. Why should we use only one solvent when preparing our samples?	The Geno-Lab bacteria perform differently in different solvents at various concentrations. Therefore, only one solvent should be used for all of the collected samples because the Geno-Lab bacteria performs differently in different solvents at various concentrations. Thus, using only one solvent will provide results that can be comparable when analyzed.
3. What are the purposes of the controls?	The above-mentioned controls were included to ensure that the kit is functioning properly. The wells in column 1 should contain a series of 2-fold dilutions of the positive control (4-NQO). A blue colour should appear with the darkest blues appearing at the top of the column with different densities corresponding to the concentration of the compound. The wells in column B are used as negative control wells containing diluent and bacteria but NO samples. Small amounts of colour change will be produced in accordance with background levels of enzyme production in the absence of induction. The wells in row H receive diluent or samples and NO bacteria. These wells serve as the reagent or sample control wells for the assay.
4. Describe what occurs during the last phase of the Geno-Lab. What is used to measure enzymes produced? How is cell survival assessed?	During the two hour incubation, genotoxic materials interact with the DNA of the Geno-Lab bacteria and induce the de novo synthesis of β galactosidase. At the last stage of the Geno-Lab, the relative amount of enzyme produced as a result of system induction, is measured by addition of a chromogenic substrate. Since the success of analysis and production of β galactosidase depend upon bacterial viability during the test, cell survival (ATP activity) is assessed using alkaline phosphatase. In this way, both acute bacterial toxicity as well as genotoxicity of the samples can be measured simultaneously.

Student Lab Report

Group #	Group Student Names	Role

Checkmark the appropriate Stream below:

Water 	Chemical 	Soil 	Synthetic 

Note: At the beginning of class, your teacher will have the lab stations ready. Remember to check your lab station, ensure you have the appropriate supplies, and read over the safety protocols.

Pre-Lab Notes	

Instructions: Read over the preparation and the procedure for the specific biotechnology kit that you will be working on. Complete the lab and record the results.

Preparation – Geno-Lab™

To Students: Please follow the instructions and information below to help prepare for the lab experiment.

Expectations

The Geno-Lab™ high school biotechnology kit is based on genetically engineered E.coli, which measures the primary response of a cell to genetic damage. The kit will provide a clear and completely objective measurement of the genotoxicity of a sample. Once your group completes the experiment, your group will record the results from your sample and then record the results of the other groups. Each group will determine genotoxicity levels and complete a lab report for assessment and evaluation.

Collecting the Samples

Your educator will discuss whether your group will need to acquire samples or if your educator will provide the samples. Four samples are required for your group to conduct this lab. The samples should consist of chemical substances from home such as but not limited to pharmaceutical products (i.e. liquid based) and outdoor products (i.e. plant food, etc.). Ensure that you understand and follow safety measures when your group acquires the samples. There should be a total of 24 samples organized as follows:

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
- Sample 1	- Sample 5	- Sample 9	- Sample 13	- Sample 17	- Sample 21
- Sample 2	- Sample 6	- Sample 10	- Sample 14	- Sample 18	- Sample 22
- Sample 3	- Sample 7	- Sample 11	- Sample 15	- Sample 19	- Sample 23
- Sample 4	- Sample 8	- Sample 12	- Sample 16	- Sample 20	- Sample 24

Lab Station Preparation

At the beginning of the lab, you should have the following available in the front of the classroom and lab station:

FRONT OF THE CLASSROOM

For use by all Student Groups:
- OD_{600} Bacterial Suspension OR SOS Express Bacterial Strain
- Refrigerator (if available)
- Incubator (if available)

GROUP LAB STATION

For each Student Group:
- Microplate
- Geno-Lab diluent (50 uL)
- 4-NQO positive control (20 uL)
- Prepared sample dilutions (4 x 20 uL)
- Blue chromogen (6 mL)
- (?) volume of bacterial suspension in a clean vial or test tube (complete volume to 10 mL with fresh medium)

Before starting an experiment it is important to ensure all of the materials, supplies and equipment needed are available and ready. Be sure to speak to your educator if there is anything missing.

Preparation of the Samples

Your educator will let you know if you are able to complete this. This will need to be done before you begin your experiment. Only one solvent should be used for all samples, controls, and blanks. This is provided in bottle (C) which consists of 10% dimethyl sulfoxide (DMSO) in sterile 0.85% saline. Do NOT use any other solvent.

See the next page for further details of how to prepare the lab experiment controls and your samples.

Positive Controls	Solid Samples	Liquid Samples	Reaction Blanks
Bottle (D) contains a 10 ug/mL solution of 4-nitroquinoline-1-oxide (4-NQO) in a microfuge tube. This will be the first dilution to be used in the standard plot. Prepare six additional two-fold serial dilutions in 10% DMSO (C).	Dissolve your sample to be tested in water (or if insoluble in DMSO) in concentrations of 100-1,000 ug/mL or higher. Prepare at least 14 two-fold dilutions in 10% DMSO saline (C).	Prepare six two-fold dilutions in (C).	Use 10% DMSO saline (C).

Ensure that you review the Lab Safety Protocols before beginning the experiment.

Storage:

- The kit components should be stored under refrigeration (2°C to 8°C) or in a freezer (-4°C to -20°C) in total darkness if possible.
- If there is repeated freeze-thaw sequences and prolonged light exposure, the 4-NQO will degrade and affect the results.
- Light is a known DNA damaging agent, and the Geno-Lab™ bacterial strain is sensitive to it. Excess light will induce the colour reaction and increase background levels of induction. KEEP EXPOSURE TO LIGHT (especially shorter wavelengths) TO A MINIMUM.

Handling:

- Handle the kit and tested samples like any potentially hazardous material.
- Collect all used components upon completing the assay and properly dispose according to laboratory protocols.
- Aseptic techniques should be employed when rehydrating the lyophilized bacteria. Cleanliness and aseptic techniques are strongly recommended for all laboratory procedures that involve bacteria.
- Note that the bacterial strain is not a known pathogen.

Results & Analysis – Geno-Lab™

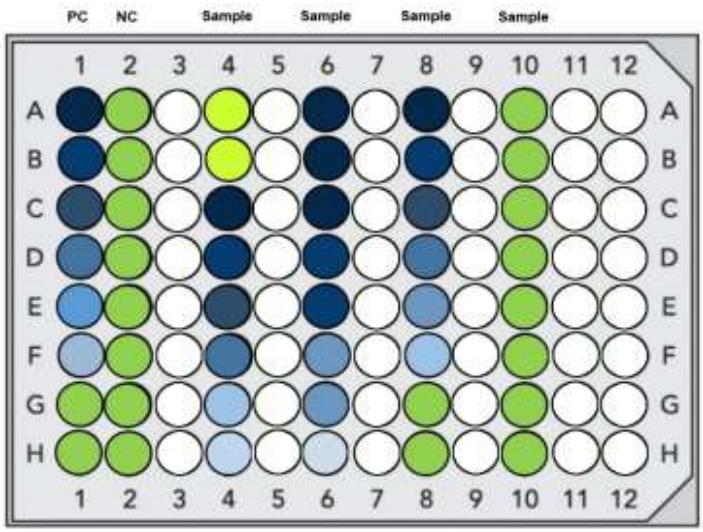
Instructions: Once you obtained the results, complete the following steps below. Read over the guidelines before recording your results.

If you have any errors from the experiment, please describe them here and the reasons why this occurred:

Observing the Results

Criteria for a Valid Test: For the Geno-Lab™ assay to be considered valid, the following conditions must be met:	a) **Positive Control (Column 1)**: The wells in column 1 should contain a series of 2-fold dilutions of the positive control (4-NQO). A blue colour should appear with the darkest blues appearing at the top of the column with different densities corresponding to the compound concentration. There should be no blue colour development or concentration gradient in the positive control column (column 1). If this occurs, the results obtained are invalid. b) **Negative Control (Column 2)**: The wells in column 2 are used as negative control wells containing diluent and bacteria but NO samples. Small amounts of colour change will be produced in accordance with background levels of enzyme production in the absence of induction. If there is no colour production in these wells, the bacteria are not viable and have died.

Sample Results of a Geno-Lab™:



Please see to the left for a guideline in how your results should look like. Please note that your results will not look exactly like this.

Figure 1: Sample Geno-Lab™ Results

Components
- Column 1: Positive control (4-NQO)
- Column 2: Negative control that contain Geno-Lab™ diluent and bacteria only
- Columns 4, 6, 8, and 10: Unknown samples diluted in each well down the column

Results Guidelines:
- Sample 1 in column 4 is genotoxic at lower concentrations (blue wells) and cytotoxic at the highest concentrations (yellow wells).
- Sample 2 in column 6 is evidently genotoxic and seem to demonstrate a dose response relationship (blue wells, gradually getting lighter).
- Sample 3 in column 8 is also evidently genotoxic and seem to demonstrate a dose response relationship (blue wells, gradually getting lighter).
- Sample 4 in column 10 is not genotoxic at all (green wells correspond to background expression of alkaline phosphatase and β-galactosidase).

Recording the Results

1. With your group, record your four sample observations in the 'Geno-Lab™ Results Table.' Record the blue colour intensity of the tested material giving reactions and assign values to them (see below for a comparative chart). Compare with values obtained for the 4-NQO for reference (using a digital photo) to other materials you may have tested or will test in the future.

The following is a checklist for your group to follow when recording results. Use the sample results (Figure 1 – previous page) as a visual guideline:

a) Check the positive 4-NQO control (column 1).
b) Check the **blue** colour density appearing in the wells of your test materials and continuously compare with the diluent-only wells.
c) Start checking from the highest concentration of the test material to the lowest.
d) High concentrations may not induce any positive response due to acutely toxic concentrations in which the cells are killed outright.
e) As the material is diluted out, toxicity is reduced and a positive reaction (**deep blue** colour) may then appear indicating chronic genotoxicity.

The colour density will fade into the background **yellow-blue** as diluted concentrations fall below genotoxic levels.

When you analyze your results, please note below:

Visual Results

The degree of genotoxicity levels will be classified into five categories based on a visual inspection of the plate wells of your samples that were tested:

(-)	Yellow-blue colour present and no genotoxicity present
(+/-)	Light blue & yellow-blue colouring indicating low genotoxicity levels
(+)	< 50% blue colour intensity compared to the control, moderate genotoxicity levels
(++)	< 100% but > 50% colour intensity compared to the control, high genotoxicity levels
(+++)	Blue colour that is equivalent to positive control and very high genotoxicity levels

Comparisons should be made to the average blue development from the positive control column (Column 1). See the left side.

Please read below as well to assist with the recording of the results:

No positive blue colour: Check the yellow colour density appearing in the wells of your test materials, following the addition of alkaline phosphatase and subsequent incubation.	**The presence of Yellow colour**: If the yellow colour appearing in the wells of the test material is similar to the background (diluent-only wells), the material was not toxic and not genotoxic.	**Yellow in the diluent control**: Try higher dilutions (lower concentrations) of test material so that inherent acute toxicity will not be expressed.

2. Once this occurs, go to the other group results and record those results within the 'Geno-Lab™ Results Table.' Compare the amount of colour development in each sample dilution. Please note that the colour development with the other samples will vary depending on the type of samples that were used.

Geno-Lab™ Results Table

Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)
1	1 (A)		2	1 (A)		3	1 (A)	
	2 (B)			2 (B)			2 (B)	
	3 (C)			3 (C)			3 (C)	
	4 (D)			4 (D)			4 (D)	
	5 (E)			5 (E)			5 (E)	
	6 (F)			6 (F)			6 (F)	
	7 (G)			7 (G)			7 (G)	
	8 (H)			8 (H)			8 (H)	

Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)
4	1 (A)		5	1 (A)		6	1 (A)	
	2 (B)			2 (B)			2 (B)	
	3 (C)			3 (C)			3 (C)	
	4 (D)			4 (D)			4 (D)	
	5 (E)			5 (E)			5 (E)	
	6 (F)			6 (F)			6 (F)	
	7 (G)			7 (G)			7 (G)	
	8 (H)			8 (H)			8 (H)	

Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)
7	1 (A)		8	1 (A)		9	1 (A)	
	2 (B)			2 (B)			2 (B)	
	3 (C)			3 (C)			3 (C)	
	4 (D)			4 (D)			4 (D)	
	5 (E)			5 (E)			5 (E)	
	6 (F)			6 (F)			6 (F)	
	7 (G)			7 (G)			7 (G)	
	8 (H)			8 (H)			8 (H)	

Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)
10	1 (A)		11	1 (A)		12	1 (A)	
	2 (B)			2 (B)			2 (B)	
	3 (C)			3 (C)			3 (C)	
	4 (D)			4 (D)			4 (D)	
	5 (E)			5 (E)			5 (E)	
	6 (F)			6 (F)			6 (F)	
	7 (G)			7 (G)			7 (G)	
	8 (H)			8 (H)			8 (H)	

Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)
13	1 (A)		14	1 (A)		15	1 (A)	
	2 (B)			2 (B)			2 (B)	
	3 (C)			3 (C)			3 (C)	
	4 (D)			4 (D)			4 (D)	
	5 (E)			5 (E)			5 (E)	
	6 (F)			6 (F)			6 (F)	
	7 (G)			7 (G)			7 (G)	
	8 (H)			8 (H)			8 (H)	

Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)
16	1 (A)		17	1 (A)		18	1 (A)	
	2 (B)			2 (B)			2 (B)	
	3 (C)			3 (C)			3 (C)	
	4 (D)			4 (D)			4 (D)	
	5 (E)			5 (E)			5 (E)	
	6 (F)			6 (F)			6 (F)	
	7 (G)			7 (G)			7 (G)	
	8 (H)			8 (H)			8 (H)	

Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)
19	1 (A)		20	1 (A)		21	1 (A)	
	2 (B)			2 (B)			2 (B)	
	3 (C)			3 (C)			3 (C)	
	4 (D)			4 (D)			4 (D)	
	5 (E)			5 (E)			5 (E)	
	6 (F)			6 (F)			6 (F)	
	7 (G)			7 (G)			7 (G)	
	8 (H)			8 (H)			8 (H)	

Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)	Sample	Dilution	Response (-, +/-, +, ++, +++)
22	1 (A)		23	1 (A)		24	1 (A)	
	2 (B)			2 (B)			2 (B)	
	3 (C)			3 (C)			3 (C)	
	4 (D)			4 (D)			4 (D)	
	5 (E)			5 (E)			5 (E)	
	6 (F)			6 (F)			6 (F)	
	7 (G)			7 (G)			7 (G)	
	8 (H)			8 (H)			8 (H)	

Further Analysis: Complete the following questions.

Explain how genotoxicity can be harmful to our bodies.

Why should we use only one solvent when preparing our samples?

What are the purposes of the controls?

Describe what occurs during the last phase of the Geno-Lab™. What is used to measure enzymes produced? How is cell survival assessed?