MUTA-ChromoPlate™ Basic Kit

Users Guide

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Kit Contents:

MUTA-ChromoPlate™ Basic Kit

Standard Reagent List (TA100)

- A: Davis-Mingoli salts (concentrate)
- B: D-glucose
- C: Bromocresol Purple
- D: D-Biotin
- E: L-Histidine
- F: Sterile distilled water
- G: Growth Media
- V: Reagent V

DMSO
Basic Ames strain: TA100 *Salmonella Typhimurium*
Sodium Azide

Disposable list

- 96-well plate (12 units)
- Reagent boats (10 units)
- Sterile 50 ml tubes (12 units)
- A membrane filter (0.22 µm)
- Zip-lock bags (two units)
- A disposable biohazard bag

Required Equipment

- Micropipette using disposable sterile tips in the range of 5 µl to 1000 µl
- An eight-channel multi-pipette (50 to 200 µl)
- Pipette Aid (Pipette gun) using 10 ml sterile pipette tips
- Vortexer (optional)
- A 37˚C incubator

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Applications:

MUTA-ChromoPlate™ Basic Kit

- Testing of industrial effluents for presence of possible mutagenic compounds.
- Screening of municipal discharges for spill contamination, improper chemical disposal.
- Routine monitoring of waste water effluent for quality and mutagenicity.
- Screening of recycled potable water supplies for presence of priority pollutants and genotoxins.
- Screening air particulate mater (PM) for sub chronic human health effects.
- Evaluating water and soil samples for elevated levels of personal care product (PCP) residues.
- Effective teaching tools for University and College laboratories to demonstrate concepts like metabolism, carcinogenesis and mutation.
- Understanding the effects of genomic integrity with xenobiotcs and promutagens.

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Standard Ames Strains:

MUTA-ChromoPlate™ Basic Kit

Strain	Type of Reversion mutation	Standard mutagen	Comment
***Salmonella typhimurium*: Histidine dependent**			
TA97a	Frameshift	9-AA or ICR191	Derived from TA1537 and includes plasmid pKM 101 which induces error-prone DNA repair enzymes to increase sensitivity
TA98	Frameshift	2NF	Derived from TA1538 and includes plasmid pKM 101 which induces error-prone DNA repair enzymes to increase sensitivity
TA100	Base-pair substitution, oxidative	4NQO	Derived from TA1535 and includes plasmid pKM 101 which induces error-prone DNA repair enzymes to increase sensitivity
TA1535	Base-pair substitution, oxidative	4NQO	uvrB repair deficient, rfa mutation increases permeability to mutagens. Sensitive to 3 unique mutagens compared to TA100 (acetaldehyde oxime, 6-mercaptopurine and 1,3-butadiene)
***Escherichia coli*: Tryptophan dependent**			
E. coli WP2 uvrA (pKM101)	Base- pair substitution	4NQO	uvrA deletion mutation eliminates accurate excision repair mechanism, contains plasmid pKM 101 which induces error-prone DNA repair enzymes and increases sensitivity

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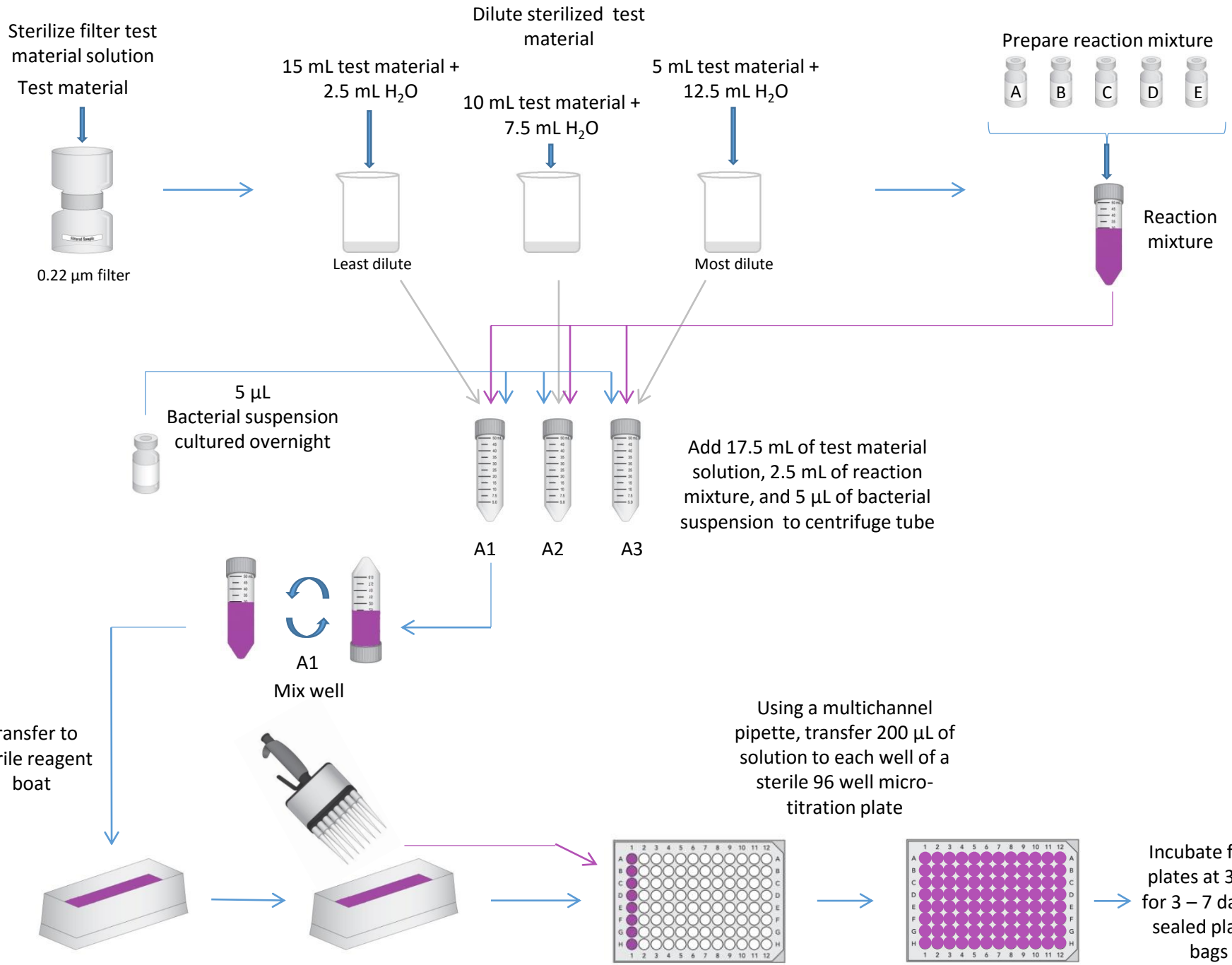

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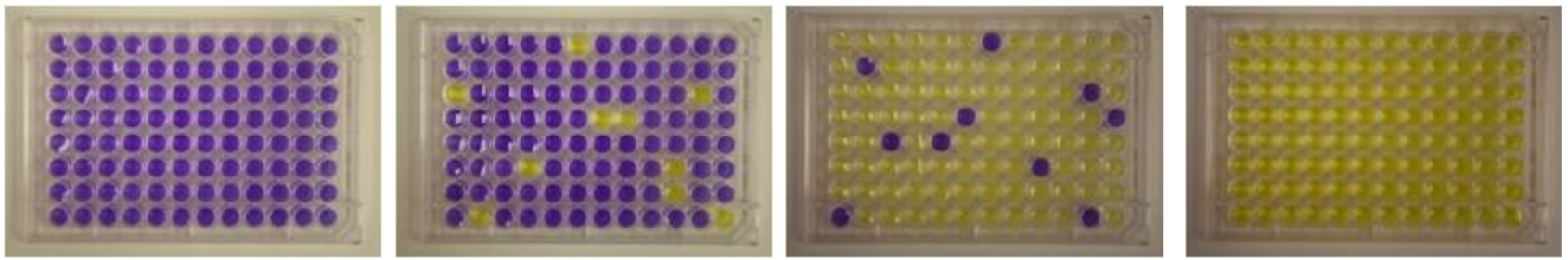
Muta-ChromoPlate™ Advantages

MUTA-CHROMOPLATE™ Basic Kit

- Reagents, bacteria and other consumable components are supplied ready-to-use in a non-specialized laboratory
- Assay endpoints are easy to read colorimetric changes that require no specialized training
- Easy to run assay (easiest Ames Test on the Market)
- Allows for larger sample volumes to be tested (up to 17.5ml of sample per plate)
- Highly sensitive to low mutagen concentrations
- Ideal for quick screening of water effluent samples



Example result plates shown above

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Bioactivation (S9 kit add-on)

MUTA-ChromoPlate™ Basic Kit

- Many mutagens must first be metabolized into their reactive form by enzymes
- Depending on the compound under study, bioactivation may be required for detection
- EBPI offers traditional methods of pro mutagen activation through the addition of S9 liver fraction
- EBPI employs a commonly used metabolic activation system which includes post-mitochondrial liver fractions isolated from Sprague Dawley rats, supplemented with cofactors
- The rats are pre-treated with Aroclor 1254 to stimulate enzyme production prior to liver extraction
- This option is offered with all mutagenicity and genotoxicity testing kits for a supplemental cost

S9 Kit Contents

S9A: $MgCl_2$ + KCl solution 0.4 mL (1 unit)
S9B: Glucose-6-phosphate solution 80 µL (1 unit)
S9C: NADP solution 250 µL (1 unit)
S9D: Phosphate buffer 1.5 mL (1 unit)
S9F: Lyophilized S9 fraction

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Experimental Overview

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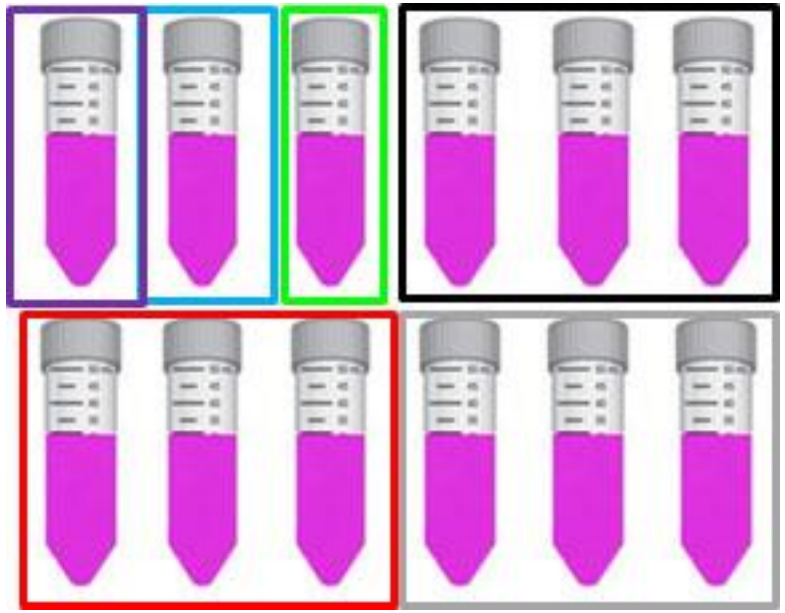
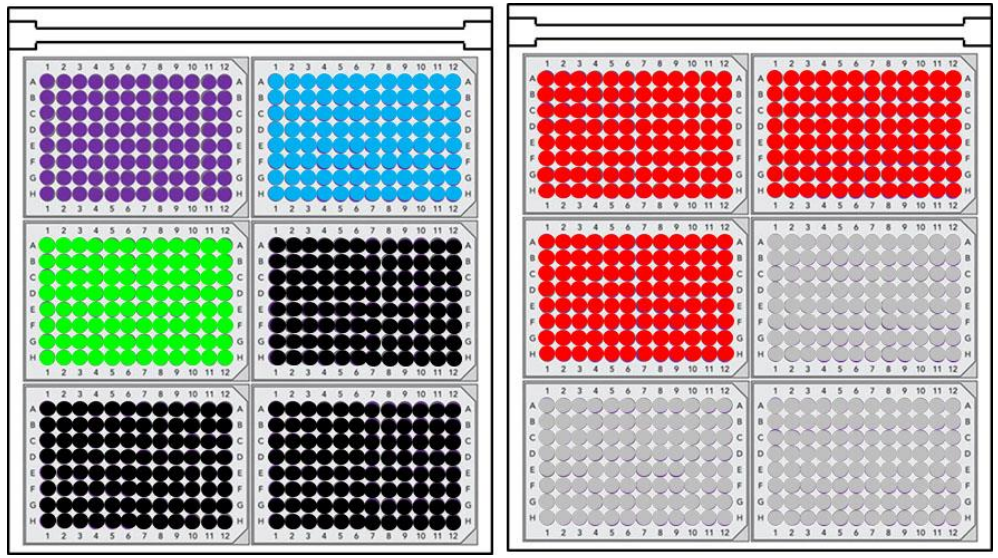
- Sterility control
- Negative control
- Positive control
- Sample 1
- Sample 2
- Sample 3



(Reversion tubes)

20 ml



12 X (96 well plate)

Note: Prior to using our test kits, we highly recommend the development of individual outlines that are representative of the respective experiment. This outline is only provided as a guideline for one possible method

The image above is an example of a test that utilizes three samples in a triplicate manner.

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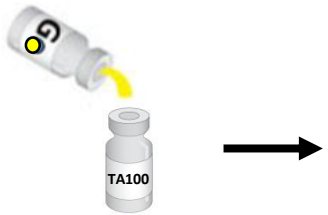
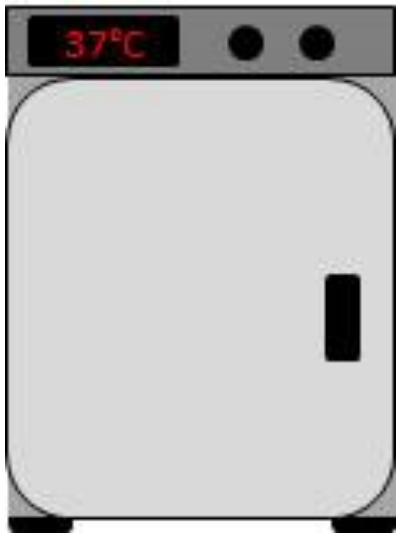
Detailed Procedure

1. Overnight inoculation and initial bacterial growth

- Always use aseptic techniques for all steps in this procedure
- Transfer growth media to bacterial bottle
- Dissolve and place in incubator overnight at 37 °C.



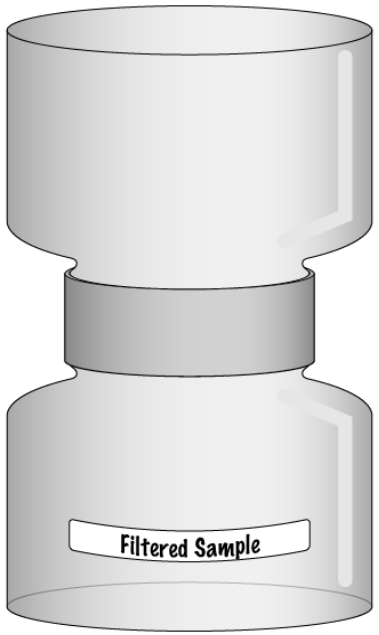
Pour media into lyophilized vial



Incubate at 37°C overnight (12-16 hrs)

2. Sample preparation

- Filter sterilize the sample to be tested using a 0.22 µm membrane filter.
- Prepared sample dilutions, in sterile distilled water, in a 50 ml sterile tubes supplied in kits
- Volume of each tube should be 17.5 ml (combination of sample and sterile distilled water).



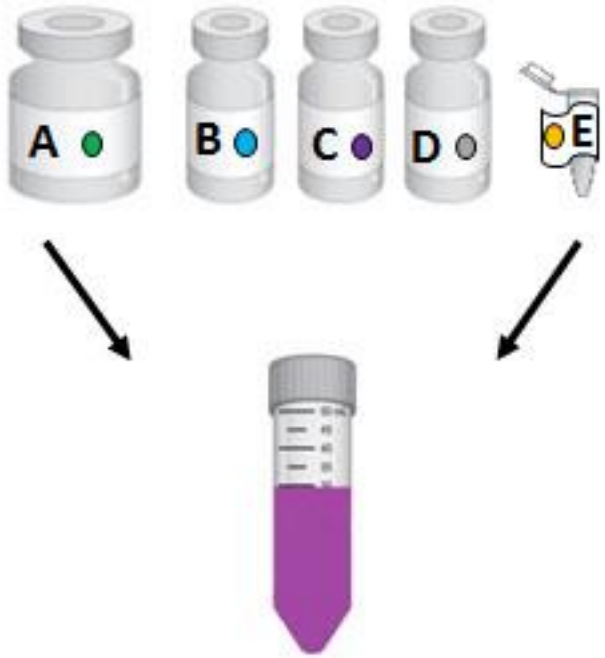
Filter sterilize samples using filter unit (large volume) or syringe filter (small volume).

3. Reaction Mix preparation

- Prepare exposure media according to guidelines below
- Ensure final solution is well mixed before continuing to next step

Master Reaction Mix

(A)		21.62 ml
(B)		4.75 ml
(C)		2.38 ml
(D)		1.19 ml
(E)	+	0.50 ml
Total		30.00 ml

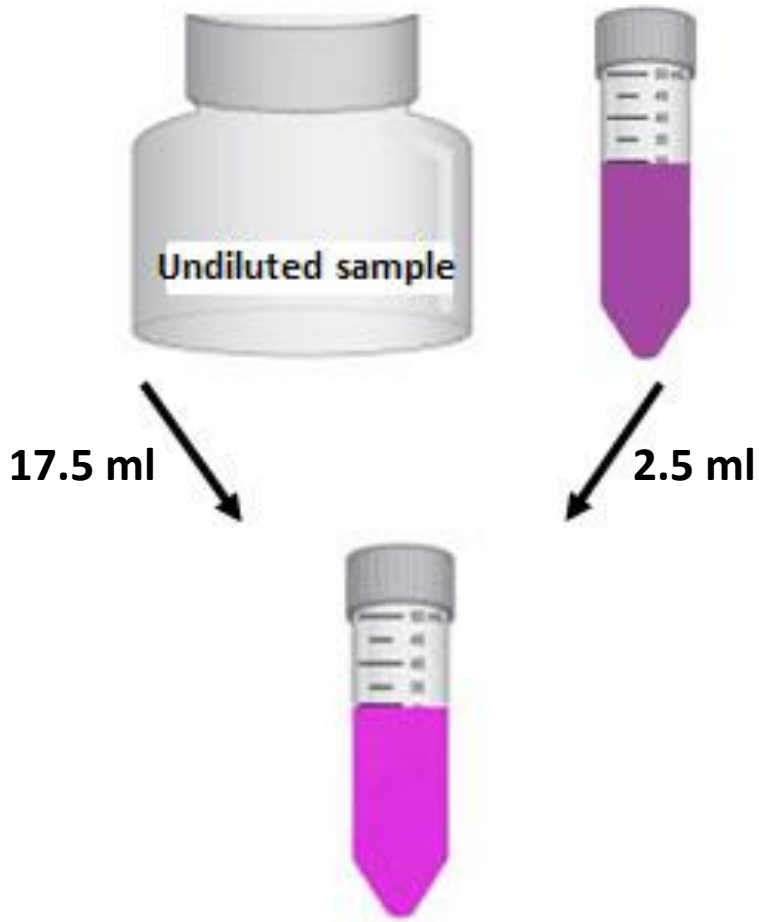


4. Add Reaction Mix to positive control tubes

Aseptically dispense 2.5 ml of the `Reaction Mix' to positive control sterile tube. Also, add 0.1 ml of sodium azide 17.4 ml of sterile water into the positive control tube.

5. Add Reaction Mix to sample tubes

Aseptically dispense 2.5 ml of the `Reaction Mix‘ to each sterile tube that will be used to contain a sample to be tested. To each tube then add **17.5 ml of the sterile filtered material to be tested**



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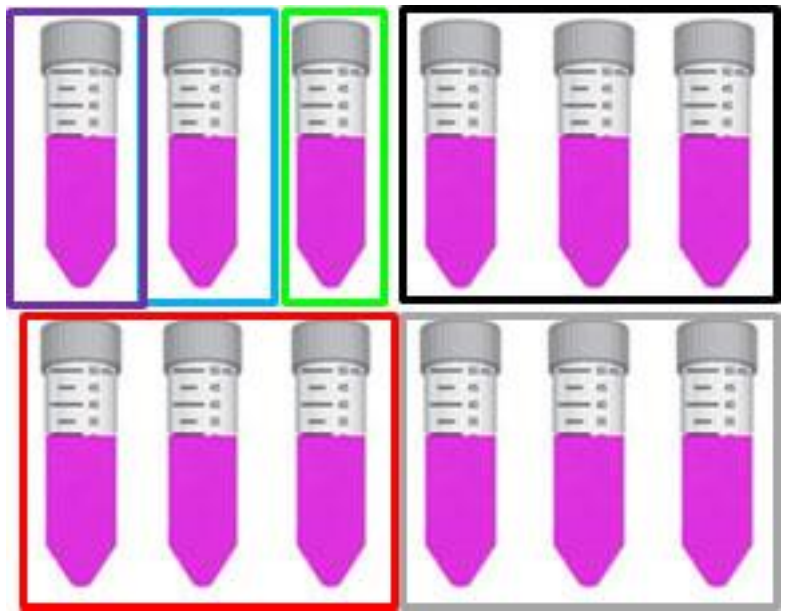
6. Transfer bacteria into sterile tubes, **except sterility control tube**

- Sterility control
- Negative control
- Positive control
- Sample 1
- Sample 2
- Sample 3



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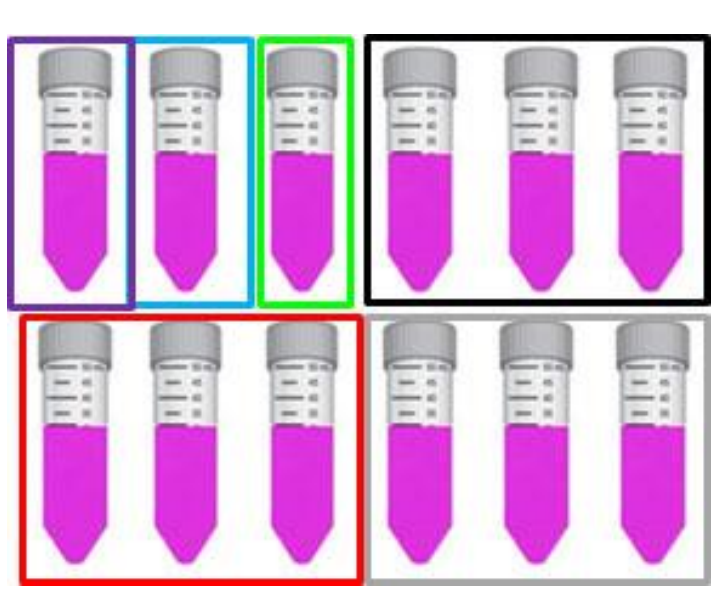
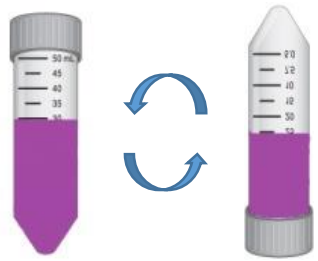
7. Transfer bacteria-reversion mix into 96-well plate

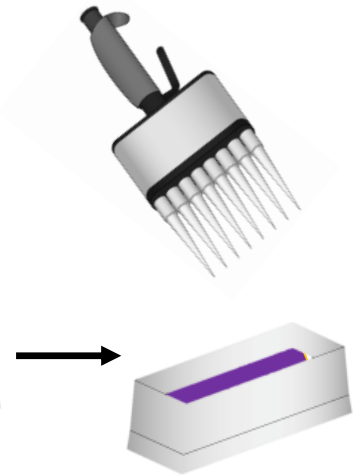
- Transferring 200 µL of bacteria-reversion mix using a multichannel pipette
- Each sample is pipetted into 96 well plate to observe reversion
- Each plate can accommodate 1 samples

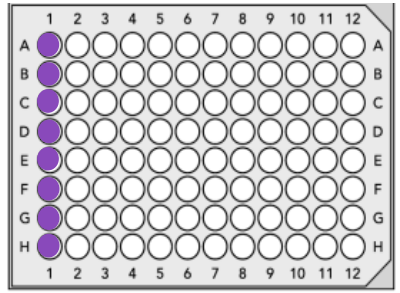




Mix

Once mixed, dump the sample into a **sterile reagent boat**

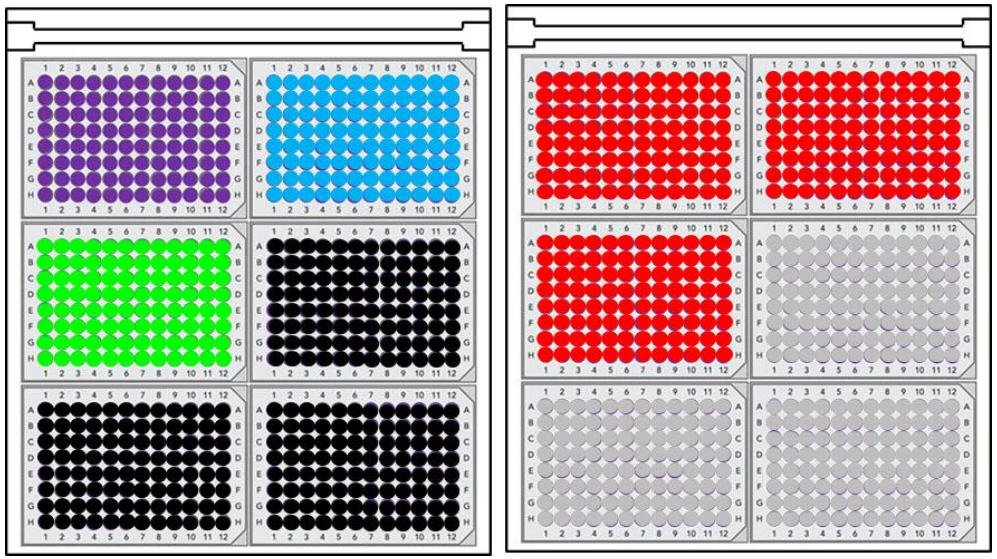




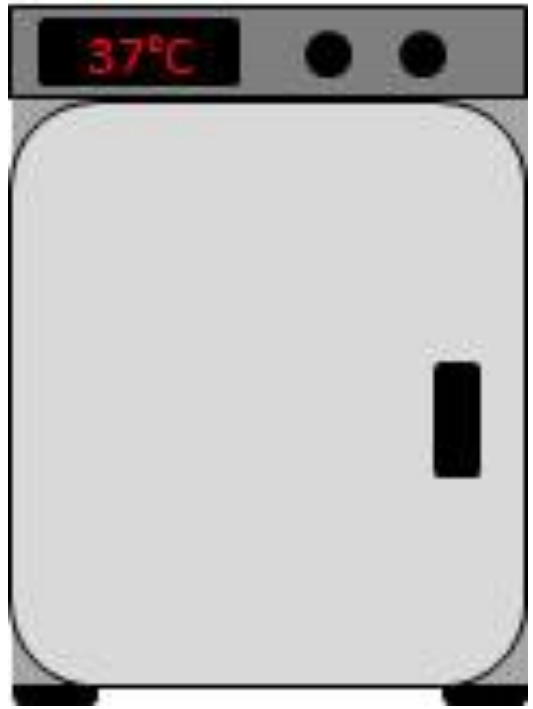
10. Incubation of 96 well plate

- Incubate 96-well plates at 37 °C for 3 - 6 days
- After three to six days, remove plates and observe the number of wells that have changed from purple to yellow
- Ensure that negative controls have small numbers of reversions, positive controls have many revertants and reagent sterility control does not have any revertants

Sterility control
Negative control
Positive control
Sample 1
Sample 2
Sample 3



12 X (96 well plate)



Incubate at 37°C for 3-6 days

Result Interpretation

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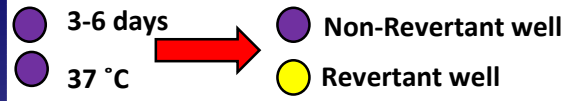

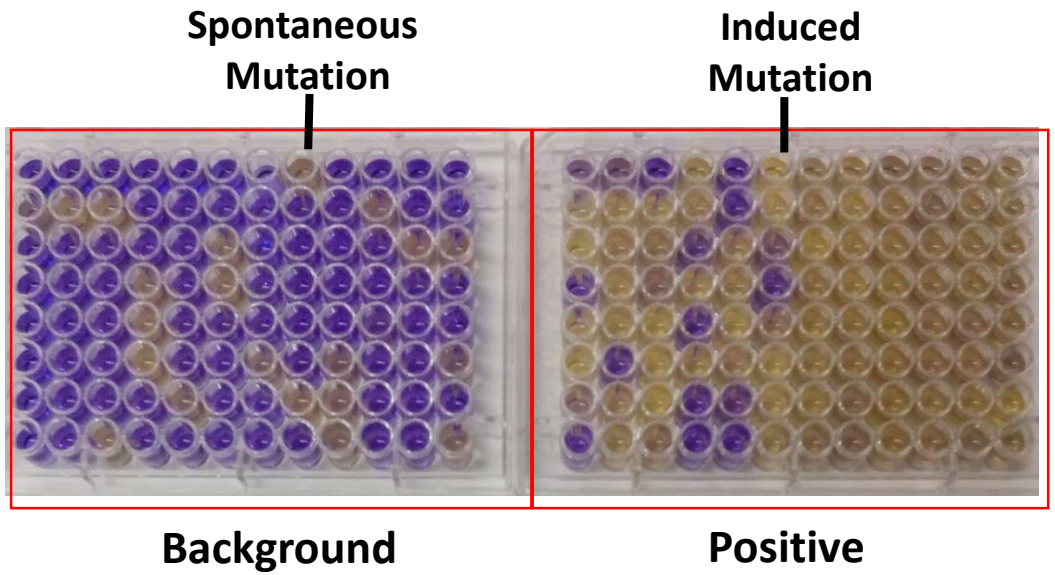

1. Plates were scored visually. Yellow and partial yellow wells are scored as positive. Purple wells are scored as negative.

2. Observe the `Blank' (sterility assessment) wells. Proceed only if the blank wells are sterile (purple). If the well is turbid or yellow, the assay may be contaminated or the sample is interfering with the reagents; results will be invalid.

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Result Significance

No. Wells Positive in Background Plate	No. Wells Positive in Treatment Plate			No. Wells Positive in Background Plate	No. Wells Positive in Treatment Plate		
	0.05	0.01	0.001		0.05	0.01	0.001
0	3	6	9	24	33	36	40
1	5	8	12	25	34	37	40
2	7	10	14	26	35	38	41
3	9	12	15	27	36	39	42
4	10	13	17	28	36	40	43
5	12	15	19	29	37	40	43
6	13	16	20	30	38	41	44
7	14	18	21	31	39	42	45
8	16	19	23	32	40	43	46
9	17	20	24	33	41	44	46
10	18	21	25	34	42	44	47
11	19	23	27	35	43	45	47
12	20	24	28	36	43	46	48
13	21	25	29	37	44	46	49
14	22	26	30	38	45	47	49
15	24	27	31	39	46	48	50
16	25	28	32	40	46	48	50
17	26	29	33	41	47	49	50

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- Depending of amounts of revertant wells in tested samples and revertant wells in negative controls, different levels of significance can be assigned
- Use the quick reference chart included with your procedure (left) or more advanced statistical methods to assign significance to mutagenicity results
- Use EBPIs Excel Sheets for interpretation and graphing of data.

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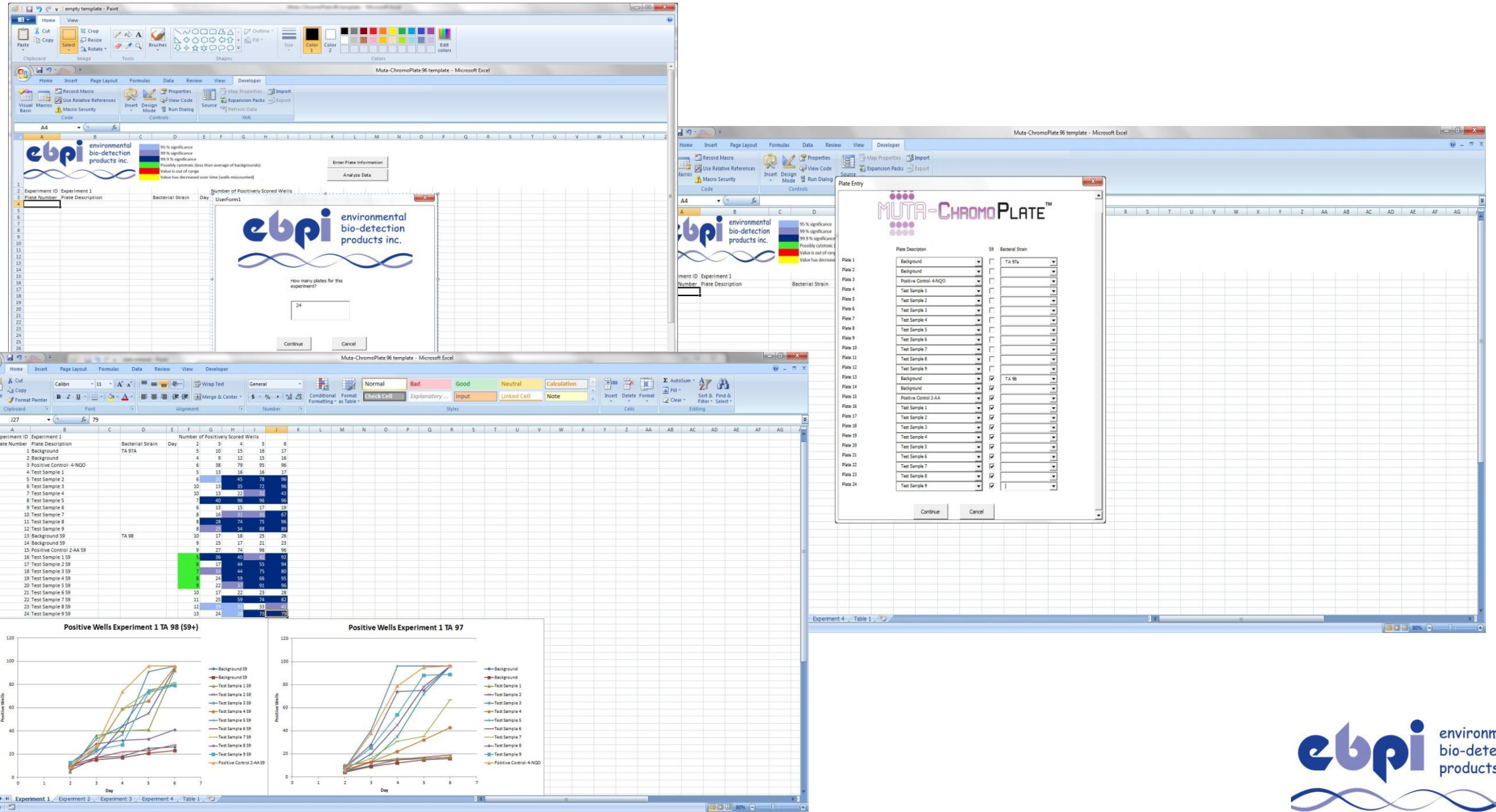
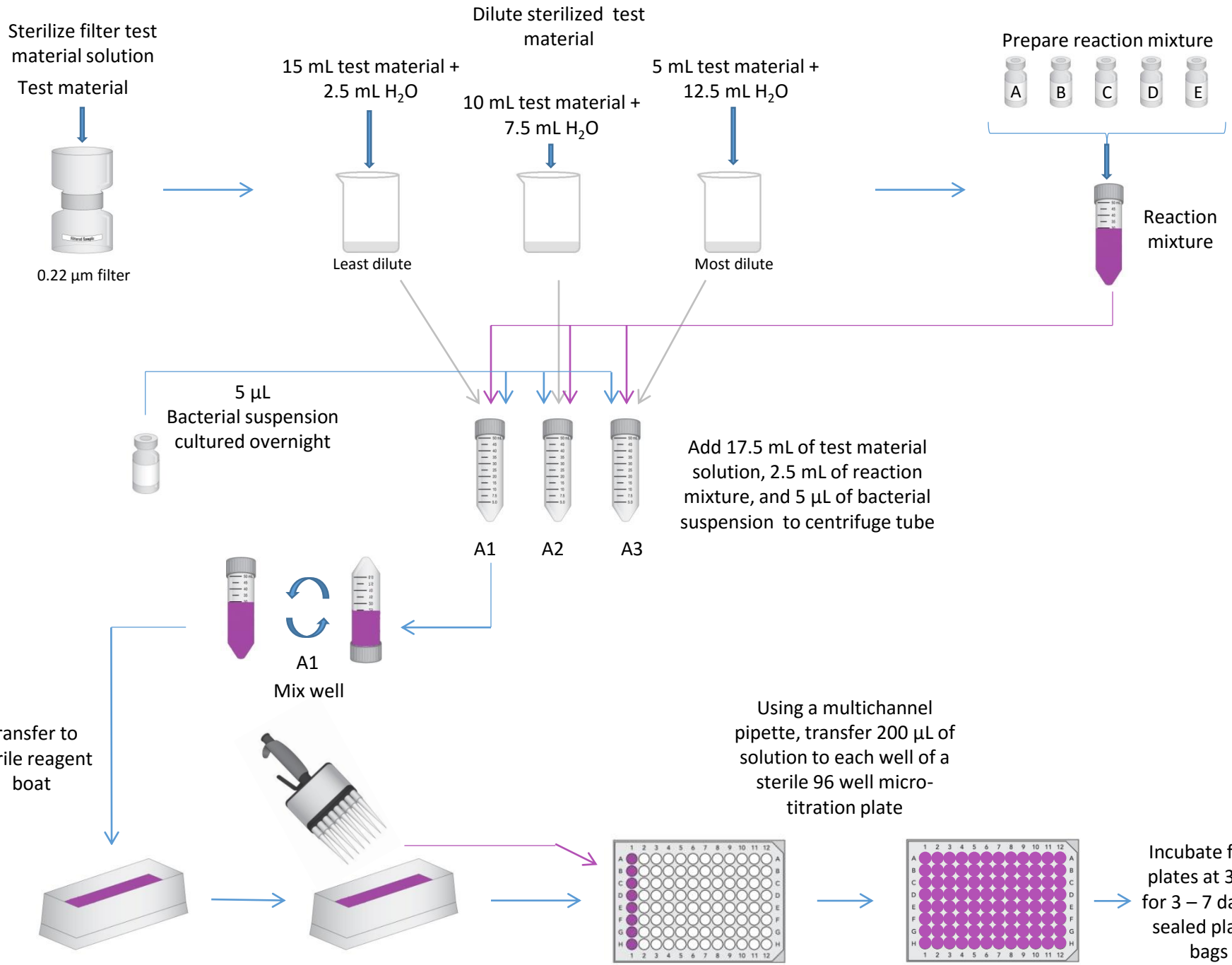
Muta-ChromoPlate EXCEL Spreadsheet





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24 Well Format

Sterilize filter test material solution

Test material

0.22 µm filter

Dilute sterilized test material

3.75 mL sample 1 + 0.625 mL H_2O

1.25 mL sample 1 + 3.125 mL H_2O

3.75 mL sample 2 + 0.625 mL H_2O

1.25 mL sample 2 + 3.125 mL H_2O

Less dilute sample 1

More dilute sample 1

Less dilute sample 2

More dilute sample 2

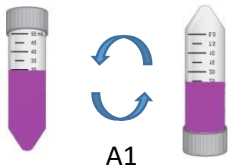
Prepare reaction mixture

Reaction mixture

2.5 µL Bacterial suspension cultured overnight

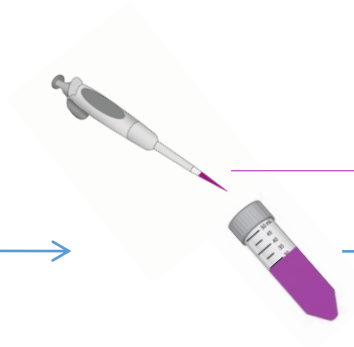
A1 A2 A3 A4

Add 4.375 mL of test material solution, 0.65 mL of reaction mixture, and 2.5 µL of bacterial suspension to centrifuge tube

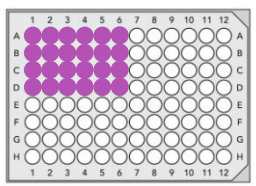


A1

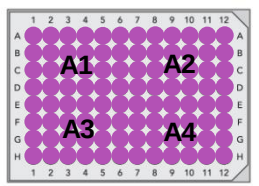
Mix well



Divide each 96-well plate into 24-well quadrants. Using a pipette, transfer 200 µL of solution A1 to each well in a quadrant.



Repeat the previous step by transferring sample A2 into a different quadrant. Repeat using samples A3 & A4 until all quadrants are filled.



Incubate filled plates at 37 ºC for 3 – 7 days in sealed plastic bags

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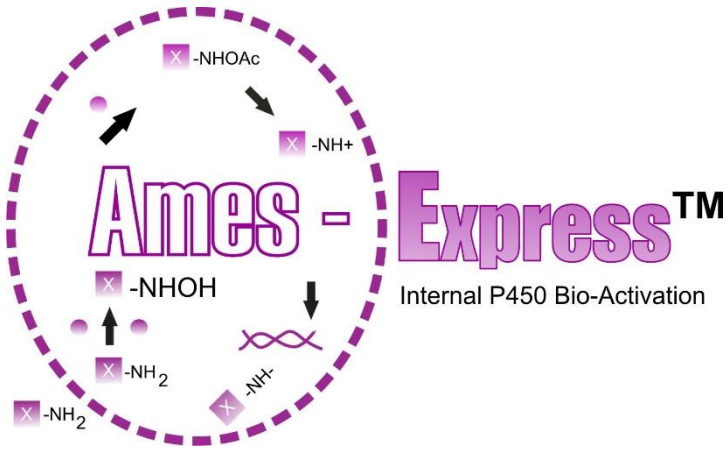
MUTA-ChromoPlate™ Basic Kit

Kit Options

- Basic Kits with and without or without S9 Activation
- Bacterial Strain Kits with or without S9 Activation
- OECD 471 Bacterial Based Kits
- Reagents Only Kits
- All kits/reagents can be modified to meet your requirements
- Ames Express Strains



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