MacroAmes1

Agar Plate Format Mutagenicity Assay

Short Protocol

For Research use only **Version 1.0 July 2023**

Please note: Items are shipped at ambient temperature with cooling elements. Kit contents will be fully active **if shipment is received within 10 days from dispatch and stored immediately as indicated on the individual items and as described on page 3-4 of this manual**. If components are damaged or if any problems occur, please contact Xenometrix by phone: ++41-61-482-14-34; fax: ++41-61-482-20-72, or via email: info@xenometrix.ch

Principle of the Test

The MacroAmes1 kit includes reagents for the bacterial reverse mutation test: Point mutations were made in the histidine *(Salmonella typhimurium)* or tryptophan *(E.coli)* operon, rendering the bacteria incapable of producing the corresponding amino acid. These mutations result in his- or trp- organisms that cannot grow unless histidine or tryptophan is supplied.

A test sample's mutagenic potential is assessed by exposing these amino acid-requiring organisms to varying concentrations of sample and selecting for the reversion event. Media lacking the specific amino acid are used for this selection which allow only those cells that have undergone the reversion to prototrophy to survive and grow. A mutagenic event causing base substitutions or frameshifts within the gene may cause a reversion to amino acid prototrophy. These reverted bacteria will then grow in histidine- or tryptophan-deficient media whereas non-reverted bacteria will not be able to grow.

The strains provided in this kit are the *Salmonella typhimurium* and *E.coli* strains that are suitable for the detection of frameshift mutations or base-pair substitutions.

The kit content and all associated reagents meet the requirements of the OECD guideline 471 for testing chemicals. [1]

Assay Description

The MacroAmes1 kit is in line with the standard Ames plate incorporation method as outlined in OECD TG471. Plating is performed into wells of a Petri dish (diameter 11 mm) containing 20–25 mL of agar. A concentration of 5000 µg/well of test compound in the MacroAmes1 kit is usually applied, depending on solubility, and as described in the regulatory document. Bacteria are exposed to 5 concentrations of a test sample, a positive and a negative control. One plate is applied for sterility testing of buffer and S9.

A dose-dependent and significant increase in the number of revertant colonies upon exposure to test sample relative to the solvent controls indicates that the sample is mutagenic in the MacroAmes1 kit.

The mutagenic potential of samples is assessed directly and in the presence of metabolic activation, provided by a rat or hamster liver homogenate, S9.

Changelog:

Date	**New version**	**Changes**
04.07.2023	1.0	• Creation of document

Genotypes of the *S. typhimurium* and *E. coli* strains

Strain	Mutation	Type	Target	Cell Wall	Repair	pKM101
TA98	*hisD3052*	Frameshift	GCGCGCGC	*rfa*	*uvrB*	✓
TA100	*hisG46*	BP substitution	GGG	*rfa*	*uvrB*	✓
TA1535	*hisG46*	BP substitution	GGG	*rfa*	*uvrB*	-
TA1537	*hisC3076*	Frameshift	GCGCGCGC	*rfa*	*uvrB*	-
E.coli* WP2 *uvrA	*trpE65*	BP substitution	CAA	-	*uvrA*	✓
Rfa	This mutation leads to a defective lipopolysaccharide (LPS) layer that coats the cell surface, making the bacteria more permeable to bulky chemicals and non-pathogenic.[2]					
uvrB/*uvrA*	The *uvrB*/*uvrA* deletion mutation eliminates the accurate excision repair mechanism, thereby allowing more DNA lesions to be repaired by error-prone DNA repair mechanisms. The deletion through the biotin gene makes the bacteria biotin dependent.					
pKM101	This R factor plasmid enhances chemical and UV-induced mutagenesis via an error-prone recombinational DNA repair pathway. The plasmid also confers ampicillin resistance.					

Kit Components and Storage Conditions

Each Xenometrix MacroAmes1 Mutagenicity Assay kit contains the following components and should be stored as indicated:

–70°C:

Vials containing bacterial strains

Note: When referring to storage at –70°C, we mean that storage at –80°C is also suitable.

Note: The bacteria are shipped with cool packs, but not frozen. Upon arrival they must be immediately stored at least at –70°C. Improper storage at –20°C may compromise the viability of the strains. The tubes are not suitable for liquid nitrogen storage.

(If no –70°C storage is available at your institution please contact Xenometrix.)

–20°C:

- Vial(s) containing ampicillin (50 mg/ml)
- S9 lyophilized

2-8°C:

- Positive controls
- Exposure Medium
- Buffers

20-25°C:

- Growth Medium
- Powder mixtures to prepare agar and soft agar

Required equipment and consumables NOT included with the kit

Note: all plasticware and glassware must be sterile!

- Environmental shaker capable of 37°C, 250 rpm incubations with approx. 2.5–3 cm amplitude. For shakers with smaller amplitude, alternative incubation vessels and rotational speeds must be validated (see section “Assay procedure day 1”). Xenometrix does not take any responsibility if bacteria do not grow due to different shaker or growth conditions
- 37°C dry incubator
- 50°C dry incubator
- 46°C dry bath or thermo block (e.g.)
- Autoclave
- Light table with magnifying glass for scoring results or automated reader (optional, recommended)
- Spectrophotometer for measuring optical density at 600 nm
- 20-µL, 200-µL, and 1000-µL adjustable pipettes and sterile tips
- Sterile 50-mL tubes with regular caps or 50-mL tubes with filter caps (or sterile cell culture flasks, small Erlenmeyer)
- Sterile 15-mL tubes with caps
- Sterile Petri Dish plates, e.g. 11 mm
- Spectrophotometer cuvettes
- Sterile 5-mL and 10-mL pipettes
- Sterile water for irrigation or for injection
- Solvents for sample dilution and solvent control (e.g., DMSO, ddH2O, …)
- Sterile S9 Co-factor solution (art.no. PCO-0800)

Safety Precautions

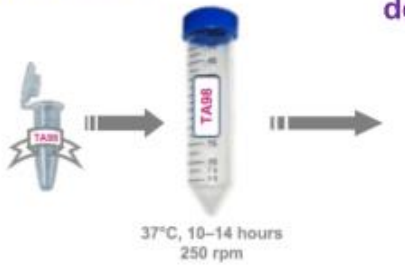
- Please consult your local guidelines for handling *S. typhimurium* and *E. coli* strains. The strains used in this kit are of low pathogenicity and are generally assigned in Risk Group Level 2. You may consult https://my.absa.org/Riskgroups for more information.
- Not for use in humans and animals. For research purposes only.
- Do not drink, eat, smoke, or apply cosmetics in designated work areas. Wear laboratory coats and gloves when handling specimens and kit reagents. Wash hands thoroughly afterwards. Do not pipette by mouth.
- Handle specimens as if capable of transmitting infectious agents. Thoroughly clean and disinfect all materials and surfaces that have been in contact with specimens. Discard all waste associated with specimens in a biohazard waste container.
- Positive control chemicals – although provided in small quantities – are mutagens/carcinogens. Please refer to the corresponding MSDS’.

MacroAmes1 Mutagenicity Assay Kit by Xenometrix

Summary of the procedure

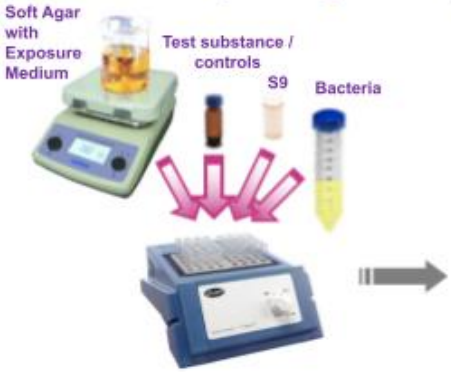
Plate and Soft Agar Preparation (Anytime prior to the experiment)

Start bacterial culture (DAY1)



Measurement of the optical density of the O/N cultures (DAY2)

Mixing of components and pouring on the plates (DAY2)



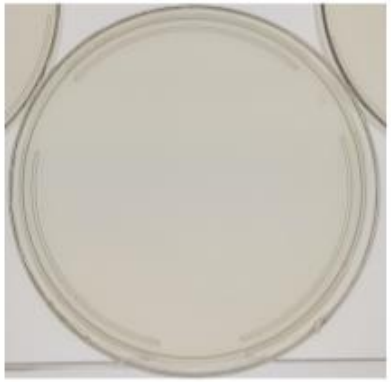


Plate scoring (DAY5)