MacroAmes1 - 98/100

Agar Plate Format Mutagenicity Assay
Without reagents for minimal glucose plates

2-Strain Version
***S. typhimurium* TA98, TA100**

Short Protocol

This protocol is a shortened version of the instruction for use for the following kit:

Art. No. W02-210-S2-P – 2 Sample Kit

Note 1

- 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 4 of this manual.**
- **No additional freeze thaw cycles of the tester strains during transportation must occur!**
- If components are damaged please contact Xenometrix by phone: +41-61-482-14-34 or by Email: info@xenometrix.ch within 3 days after receipt of product.
- This is a bioassay and these Instructions for Use must be followed strictly. Xenometrix does not take any responsibility if the Instruction for Use are not followed in detail.

- For further information please do not hesitate to contact:

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Country of Origin: Switzerland

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The complete Instruction for Use for Ames Plate Incorporation Test and Ames Preincubation Test are provided with the Kit only.
After purchase of the Kit registration on www.xenometrix.ch is available, all certificates of analysis and Instructions for Use can be downloaded from the protected “Download” area. If you are not registered to the protected area of the Xenometrix homepage, please contact info@xenometrix.ch.

Note 3

This manual applies to the following versions of the assay:

Art.No.	Number of samples [1]	Lyophilized liver S9	Positive Controls [2, 3]		
			2-NF	4-NQO	2-AA
W02-210-S2-P	2	**PB/NF [4] induced**	✓	✓	✓

[1]: Sufficient for 2 or 6 samples when tested with and without S9, in triplicates, 5 concentrations, with negative and positive controls, including sterility plates. This equals a total for 2 samples: 2 samples x 2 strains x 42 plates plus 6 sterility plates or for 6 sample Kit 3 times more. When using duplicates instead of triplicates, the number of test samples can be increased.

[2]: 2-NF: 2-Nitrofluorene; 4-NQO: 4-Nitroquinoline-N-oxide; 2-AA: 2-Aminoanthracene

[3]: Please refer to the Certificate of Analysis of each positive control's lot before using it. Please note that the MacroAmes1 98/100 is a biological assay and Xenometrix does not take any responsibility for choosing the right concentrations of the positive control.

[4]: PB/NF-induced S9: Phenobarbital/β-Naphtoflavone-induced S9.

Products available separately: S9 Cofactor Kit

Art.No.	Product	Volumes
PCO-1800	S9 Cofactor Kit: - S9 Buffer Salts pH 7.4 - S9 Buffer M - S9 G-6-P - S9 NADP	 55.0 mL 3.3 mL 2.6 mL 10.0 mL

Note 4

Please read carefully the entire manual before starting the experiments!
Xenometrix does not take any responsibility for handling errors.

1. Principle of the Test

The MacroAmes1 kit includes reagents for the bacterial reverse mutation test: Point mutations were made in the histidine (*Salmonella typhimurium*) operon, rendering the bacteria incapable of producing the corresponding amino acid. These mutations result in *his*- organisms that cannot grow unless histidine 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 histidine 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-deficient media whereas non reverted bacteria will not be able to grow.

The strains provided in this kit are the *Salmonella typhimurium* strain TA98 strain, suitable for the detection of frameshift mutations, and TA100, which detects base-pair substitutions.

The Kit content and all associated reagents meet the requirements of the OECD guideline 471 for testing chemicals.

The Kit does not contain minimal glucose agar plates. The minimal glucose agar plates must be purchased separately or prepared following the standard protocols described in the literature (Maron and Ames, 1983).

This document describes the protocol for the plate incorporation Petri dish-based Ames test. For certain chemical classes it is more suitable to perform a modified version of the plate incorporation Petri dish-based Ames test, which is referred to as pre-incubation protocol. The pre-incubation protocol is described in the Appendix.

2. Assay Description

The MacroAmes1 98/100 kit is in line with the Ames plate incorporation method in Petri Dishes as outlined in OECD TG471. Plating is performed into a Petri dish (diameter 11 cm) containing 20–25 mL of agar. A concentration of 5000 µg/well of test compound in the MacroAmes1 98/100 kit is usually applied, depending on solubility and as described in the regulatory document. Other top concentrations and dilution steps can be applied in this assay. Bacteria are exposed to 5 concentrations of a test sample, a positive and a negative control. One plate is applied for sterility testing of each buffer and S9.

A dose-dependent increase in the number of revertant colonies upon exposure to test sample relative to the solvent controls as well as a minimal fold induction of 2 of the test sample indicates that the sample is mutagenic in the MacroAmes1 98/100 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 fraction.

3. Genotypes of *S. typhimurium* Strains

Strain	Mutation	Type	Target	Cell Wall	Repair	pKM101
TA98	*hisD3052*	Frameshift	GCGCGCGC	*rfa*	*uvrB*	✓
TA100	*hisG46*	BP substitution	GGG	*rfa*	*uvrB*	✓
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.[1]					
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.					

4. Kit Components and Storage Conditions of Products Upon Arrival

Product	Art. No.	Volume or Quantity	R02-210-S2-P	R06-210-S2-P	Storage [1]
Strains **TA98** [1] **TA100** [1]	 PSS-0110 PSS-0111	 50 µL 50 µL	 1 1	 3 3	 –80°C
Ampicillin 50 mg/mL	PAM-0002	120 µL	1	1	–20°C
S9 lyophilized	PRS-PB01 or PRS-PB02	1 mL or 2 mL	4 or 2	n.a. 6	–20°C or –80°C
Positive controls [2] **2-Nitrofluorene-TA98** **4-Nitroquinoline-N-Oxide-TA100** **2-Aminoanthracene- with S9**	 PPC-NF00 PPC-NQ02 PPC-AA01	 20 µg 50 µg 100 µg	 1 1 1	 1 1 2	 2 –8 °C
Growth Medium [3]	PMM-GM00	50 mL	1	3	18 – 25°C
Mix C-1 [3]	PMN1-MSA17	1.76 g	2	6	18 – 25°C
Exposure Medium-1	PMN1-EXM35	35mL	1	3	2 – 8°C
Buffer A – 1	PMN1-BUA50	50 mL	1	3	2 – 8°C
Buffer M – 1	PMN1-BUM30	3.0 mL	1	3	2 – 8°C

[1]: Bacteria are validated for shipment at room temperature for maximum of 10 days. They are shipped with cool packs, but **must not** undergo additional freeze-thaw cycles during shipment. Upon arrival they **must** be immediately stored at least at –70°C to –80°C. Multiple freeze-thaw cycles and improper storage at –20°C may compromise the viability of the strains. The tubes are not suitable for liquid nitrogen storage. If no –80°C storage is available at your facility, please contact Xenometrix AG. Xenometrix excludes all liability for improper handling.
[2]: once dissolved, aliquot and store at –20°C.
[3]: 18°C – 25°C protected from light.

Note 5
Please contact info@xenometrix.ch if you would like to have a kit with minimal glucose plates.

5. Required Equipment and Consumables NOT Included in the Kit

- 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. Eppendorf® ThermoMixer® F2.0)
- Autoclave
- Light table with magnifying glass for scoring results or automated reader (optional, recommended)
- Spectrophotometer with cuvettes or plate reader with microplate 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 Reagent reservoirs
- 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-1800)
- 78 Petri dishes with minimal glucose agar are required for testing 2 compounds in 1 strain: 2 x 30 Petri dishes for 2 test compounds, 6 dishes for negative control (+/- S9), 6 dishes for positive control (+/- S9), 6 dishes for sterility control of buffer AM and S9 mix

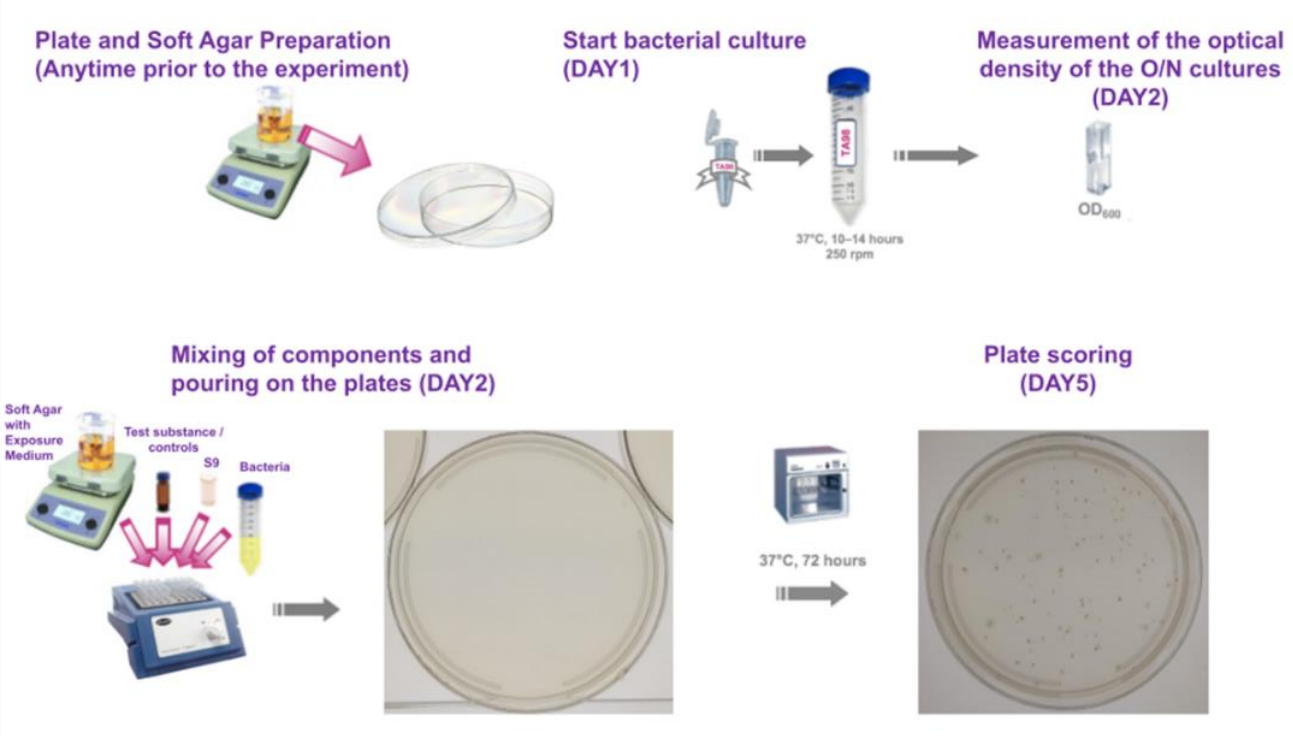
Note 6
All plasticware must be sterile. Xenometrix does not take any responsibility, if the assay is not run according to the recommendations.

6. Safety Precautions

- Please consult your local guidelines for handling *S. typhimurium* strains in the lab. The strains used in this kit are of low pathogenicity and are generally assigned in Risk Group Level 1 depending on country-specific regulations. You may check http://www.absa.org/riskgroups/bacteria.html homepage for more information.
- All kit components are not for use in humans and animals, and for Research Use Only.
- Do not drink, eat, smoke, or apply cosmetics in designated work areas. Wear laboratory coats, gloves and other necessary safety equipment when handling specimens and kit reagents. Wash hands thoroughly afterwards. Do not pipette by mouth.
- Handle specimens as if capable of transmitting infectious agents and work under a flow bench. 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. Although provided in small quantities, positive control chemicals are mutagens/carcinogens. Please refer to the corresponding MSDS.
- Xenometrix AG does not take the responsibility for any accidents or adverse human health outcomes as a result of the usage of its products other than the intended use described in this Instructions for Use document.

7. Assay Procedure

Note 7
The kit is designed for testing 2 compounds with 2 strains. Any number and combination of strains can be handled in a single experiment. In order to minimize complexity (different media, ampicillin requirements, strain dilutions, positive controls) we recommend considering carefully the number of strains and test compounds that should be tested in one single experiment. **The following text describes an experiment for 1 strain with 2 test compounds.**



8. References

[1]: Mortelmans and Zeiger. 2000. The Ames Salmonella/microsome mutagenicity assay. *Mutat. Res*. 455:29-60.
[2]: M. Kato et al. 2018 Negative and positive control ranges in the bacterial reverse mutation test: JEMS/BMS collaborative study. Genes Environ. 40: 7
[3]: D. Maron and B. Ames. 1983 Revised Method for the Salmonella mutagenicity test. Mutat. Res. 113:173-215
[4]: S. Flückiger-Isler and M. Kamber. 2012. Direct comparison of the Ames microplate format (MPF) test in liquid medium with the standard Ames preincubation assay on agar plates by use of equivocal to weakly positive test compounds: Mutation Research 747 (2012) 36– 45
[5]: Levy DD, Zeiger E, Escobar PA, Hakura A, van der Leede BM, Kato M, Moore MM, Sugiyama KI. Recommended criteria for the evaluation of bacterial mutagenicity data (Ames test). Mutat Res Genet Toxicol Environ Mutagen. 2019 Dec;848:403074. doi: 10.1016/j.mrgentox.2019.07.004. Epub 2019 Aug 5. PMID: 31708073.