MicroAmes6 – Penta 4

Ames Test in 6 Well Plates

5 – Strain Version
2 – Sample Kit

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

Art. No. Y02-514-S2-P

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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Manufactured by Xenometrix AG
Country of Origin: Switzerland

Note 2

After registration on www.xenometrix.ch, 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 version:
Sufficient for 2 samples tested in all 5 strains with and without S9, in triplicates, 6 concentrations, with negative and positive controls, including sterility plates. In total 90 x 6 Well Agar Plates can be produced.

Art. Nr.	Strains				
Y02-514-S2-P	TA98	TA100	TA1535	TA1537	E.coli uvrA[pKM101]

Products available separately: S9 Cofactor Kit

Art. No	Product	Volumes
PCO-0800	S9 Cofactor Kit: - S9 Buffer Salts pH 7.4 - S9 Buffer M - S9 G-6-P - S9 NADP	 25.0 mL 1.5 mL 1.1 mL 4.1 mL

Note 4

Please read carefully the entire manual before starting the experiments!
Xenometrix does not take any responsibility for handling errors.
Please refer to the Certificate of Analysis of each positive control's lot before using it. Please note that the MicroAmes6 Penta 4 is a biological assay and Xenometrix does not take any responsibility for choosing the right concentrations of the positive controls.

1. Principle of the Test

The MicroAmes6 Penta 4 Kit includes reagents for the bacterial reverse mutation test: Point mutations were made in the histidine (*Salmonella typhimurium*) operon or in the tryptophane (*E.coli*), rendering the bacteria incapable of producing the corresponding amino acid. These mutations result in *his*- or *trp*- organisms that cannot grow unless histidine or tryptophane 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- or tryptophane-deficient media whereas non-reverted bacteria will not be able to grow.

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

This document describes the protocol for the plate incorporation Ames test in 6 Well Agar Plate Format. For certain chemical classes it is more suitable to perform a modified version of the plate incorporation Ames Test in 6 Well Agar Plate Format, which is referred to as pre-incubation protocol. The pre-incubation protocol is described in the Appendix.

2. Assay Description

The MicroAmes6 Penta 4 kit is a miniaturized version of the standard Ames plate incorporation method, with the exception that plating is performed into wells of a 6-well plate containing 5 mL of agar (compared to a normal sized Petri dish containing 20–25 mL of agar). A concentration of 1000 µg/well of test compound in the MicroAmes6 Penta 4 kit is considered equivalent to 5000 µg/plate, *i.e.*, the concentration required in the regulatory Ames test.[1] According to the plating scheme we suggest, bacteria are exposed to 6 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 MicroAmes6 Penta 4 kit.

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

3. Genotypes of *S. typhimurium* Strains and the *E.coli* Strain

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	+1 frameshift *near C-C-C run*	*Rfa*	*uvrB*	-
E.coli WP2 uvrA[pKM101]	*trpE65*	BP substitution	A:T	-	*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.[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	Storage	Y02-514-S2-P
TA98 [1]	PSS-0110	50 µL	-80°C	1
TA100 [1]	PSS-0111	50 µL	-80°C	1
TA1535 [1]	PSS-0112	50 µL	-80°C	1
TA1537 [1]	PSS-0113	50 µL	-80°C	1
E.coli WP2 uvrA[pKM101] [1]	PSS-0119	50 µL	-80°C	1
Ampicillin	PAM-0002	120 µL	-20°C	1
S9 Lyophilized	PRS-PB01	1.0 mL	-20°C or -80°C	3
2-Nitrofluorene [2]	PPC-NF00	20 µg	2 – 8°C	1
4-Nitroquinoline-N-Oxide [2]	PPC-NQ02	50 µg	2 – 8°C	1
N4-Aminocytidine [2]	PPC-AC02	2.5 mg	2 – 8°C	1
9-Aminoacridine [2]	PPC-AR05	1.0 mg	2 – 8°C	1
2-Aminoanthracene [2]	PPC-AA01	100 µg	2 – 8°C	2
Exposure Medium-6	PMN6-EXM14	14 mL	2 – 8°C	2
E.coli Exposure Medium-6	PMN6-ECE07	7 mL	2 – 8°C	1
Buffer A-6	PMN6-BUA04	4 mL	2 – 8°C	7
Buffer M-6	PMN6-BUM06	0.6 mL	2 – 8°C	3
Growth Medium [3]	PMM-GM00	50 mL	18 – 25°C	2
Mix A-6 [3]	PMN6-MGA81	8.1 g	18 – 25°C	5
Mix B50-6 [3]	PMN6-HGA24	24 mL	18 – 25°C	3
Mix BG-6 [3]	PMN6-HGA57	57 mL	18 – 25°C	3
Mix C-6 [3]	PMN6-MSA07	0.72 g	18 – 25°C	5

[1]: Bacteria are validated for shipment at room temperature for maximum of 10 days. They are shipped with cool packs, but **must not** undergo multiple 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 without minimal glucose plates or a kit with alternative strain configuration, e.g. with Salmonella strains TA97a or TA102.

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
- Counting chamber and light microscope for the determination of the cell density
- 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, non-pyrogenic 5-mL tubes with caps
- Sterile 6 Well Plates
- 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-0800)

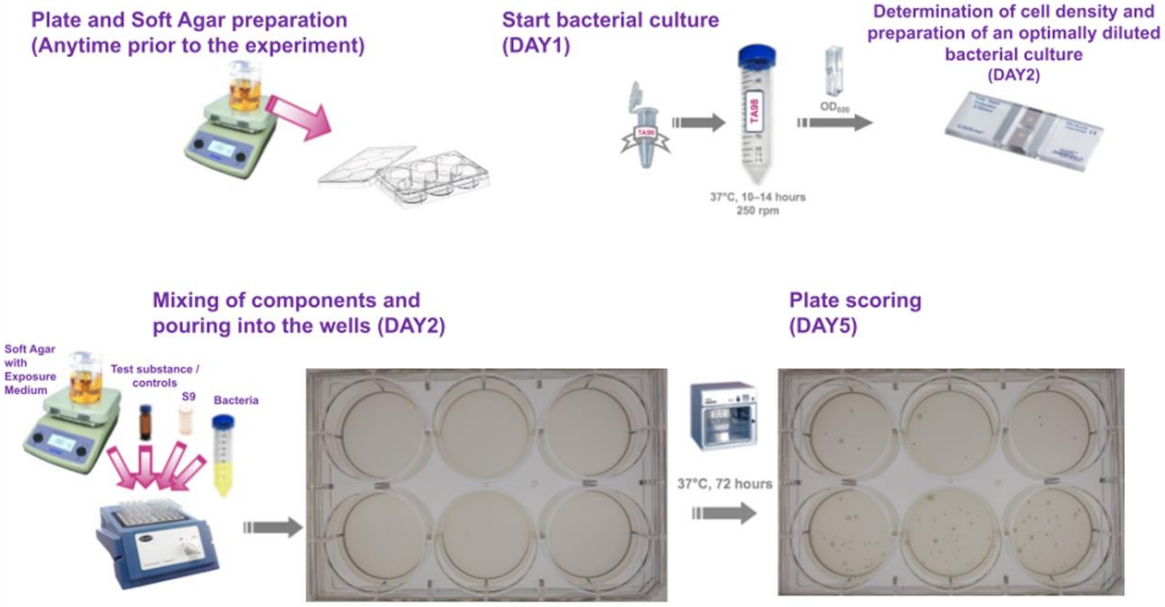
Note 6
All plasticware and glassware must be sterile. Xenometrix does not take any responsibility, if the assay is not performed according to the recommendations.

6. Safety Precautions

- Please consult your local guidelines for handling *S. typhimurium* Ames tester strains and *E.coli* Ames tester 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 the http://www.absa.org/riskgroups/bacteria.html homepage for more information.
- All kit components are not for use in humans and animals or for diagnostic use, they are 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. 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.
- Handle specimens as if capable of transmitting infectious agents and work under a flow bench if possible. 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.

Note 7
The kit is designed for testing in total 2 compounds with 5 strains. A minimal number of 2 compounds in combination of any 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.

7. Assay Procedure



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.