Ames MPF™ Penta 2

Microplate-Fluctuation Mutagenicity Assay

***S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 *uvrA*[pKM101]**

Short Protocol

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

Art. No. **B01-513-S2-P**
B10-513
B10-513-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**.
- **ALL AMES STRAINS AND THE S9 FRACTION MUST NOT UNDERGO A FURTHER FREEZE THAW CYCLE BETWEEN THE STOCK OF XENOMETRIX AND THE ENDUSER!**
- 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 Instructions 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, Instructions for Use and the excel calculation sheet can be downloaded from the protected area. The step-by-step procedure can be downloaded from the homepage www.xenometrix.ch directly.

If you are not registered to the protected area of the Xenometrix homepage, please contact info@xenometrix.ch.

Note 3

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

Art.No.	Kit size[1]	Lyophilized liver S9	Positive Controls[2]					
			2-NF	4-NQO	9-AAc	N^4-ACT	2-AA	2-AF
B01-513-S2-P	1	PB/NF-induced[3]	✓	✓	✓	✓	✓	✓
B10-513	10	–	–	–	–	–	–	–
B10-513-S2-P	10	PB/NF-induced[3]	✓	✓	✓	✓	✓	✓

1: Sufficient for 1 or 10 samples when tested with and without S9, in triplicates, 6 concentrations, with negative and positive controls. This equals a total of 48 (1 sample kit) or 480 measurements (10 sample kit) per strain.
2: 2-NF: 2-Nitrofluorene; 4-NQO: 4-Nitroquinoline-N-oxide; 9-AAc: 9-Aminoacridine; N4-ACT: N4-Aminocytidine; 2-AA: 2-Aminoanthracene; 2-AF: 2-Aminofluorene.
2: Please refer to the Certificate of Analysis of each positive control's lot before using it. Please note that the Ames MPF™ is a biological assay and Xenometrix does not take any responsibility for choosing the right concentrations of the positive control.
3: PB/NF-induced S9: Phenobarbital/β-Naphtoflavone-induced S9.

Note 4

Please note that the *Salmonella* TA98, TA100, TA1535, TA1537 and the *E. coli* WP2 *uvrA*[pKM101] use different Exposure and Indicator Media due to different amino acid requirements of the *S. typhimurium* and *E. coli* strains. Media specific for *Salmonella* strains are labelled **black**; media for *E. coli* strains are labelled **red** with a extra yellow label on the cap. The growth media (GM) is the same for all strains.

Note 5

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

Ames MPF™ Penta 2

A miniaturized bacterial reverse mutation assay

1. Principle of the Test

Point mutations were introduced into the histidine operon in *Salmonella typhimurium*, rendering the bacteria incapable of producing histidine. These mutations result in *his*- organisms that cannot grow unless histidine is supplied. When a mutagenic event occurs, base substitutions or frameshifts within the operon gene may cause a reversion to amino acid prototrophy. The reverted *Salmonella* bacteria will then grow in histidine-deficient media. Bacterial metabolism and growth reduce the pH of the medium, changing the color of that well from purple to yellow. The number of yellow wells containing revertant colonies are counted for each dose and compared to a solvent (negative) control.

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 allows only those cells that have undergone the reversion to histidine/tryptophan prototrophy to survive and grow.

The Escherichia coli strains provided in this kit carry the trpE65 mutation. This mutation results in bacteria with a trp- phenotype that cannot grow unless tryptophan is supplied. When a mutagenic event occurs, base substitutions within the Trp gene (or elsewhere, see below) may cause a reversion to tryptophan prototrophy. These reverted bacteria will then grow in tryptophan-deficient media.
A chemical's mutagenic potential is assessed by exposing these trp- organisms to varying concentrations of chemical and selecting for the reversion event. Medium lacking tryptophan is used for this selection which allows only those cells that have undergone the reversion to tryptophan prototrophy to survive and grow.
Revertants can arise from a base change at the trpE65 mutation, or alternatively, by a suppression mutation.

The strains provided in this kit are the *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and the *E. coli* strain *E. coli* WP2 uvrA[pKM101]. TA100, TA1535 and *E. coli* WP2 uvrA[pKM101] are used for the detection of base substitution mutations and TA98 and TA1537 are suitable for the detection of frameshift mutations. The *S. typhimurium* strains have GC base pairs whereas the E. coli strain has an AT base pair at its primary reversion site and detects certain oxidizing mutagens, cross-linking agents and hydrazines.

The strains included in this kit meet the requirements of the OECD guideline 471 for testing chemicals.[1]

2. Assay Description

Bacteria are exposed to 6 concentrations of a test sample, as well as a positive and a negative control, for 90 minutes in medium containing sufficient histidine (*S. typhimurium*) or tryptophan (*E. coli*) to support approximately two cell divisions. After exposure, the cultures are diluted in pH indicator medium lacking histidine (or tryptophan for *E. coli* strains), and aliquoted into 48 wells of a 384-well plate. Within two days, cells that have undergone reversion to histidine (or tryptophan) prototrophy will grow into colonies. Bacterial metabolism reduces the pH of the medium, changing the color of that well. The number of wells containing revertant colonies are counted for each dose and compared to a solvent (negative) control. Each dose is done in triplicate to allow for statistical analysis of the data and each dose is run in absence and in presence of rat liver microsomal fraction S9 to allow the compound to undergo metabolic activation.
A dose-dependent increase in the number of revertant colonies upon exposure to test chemical relative to the solvent controls indicates that the sample is mutagenic in the Ames MPF™ Penta 2 assay.

3. Genotypes of the *Salmonella 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	+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.[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.					

4. Kit Components and Storage Conditions of Products Upon Arrival

Product	Art. No.	Volume	B01-513-S2-P	B10-513	B10-513-S2-P	Storage[1]
Strain TA98	PSS-0110	250 µL[3]	1	10	10	
Strain TA100	PSS-0111	250 µL[3]	1	10	10	
Strain TA1535	PSS-0112	250 µL[3]	1	10	10	–80°C
Strain TA1537	PSS-0113	250 µL[3]	1	10	10	
Strain E.coli uvrA(pKM101)	PSS-0119	250 µL[3]	1	10	10	
Ampicillin 50 mg/mL	PAM-0002	120 µL	1	3	3	–20°C
S9 lyophilized[4]	PRS-PB01	1 mL	2	-	-	–20°C or
	PRS-PB02	2 mL	-	-	10	–80°C
Positive controls[5]						
2-Nitrofluorene	PPC-NF00	20 µg	1	–	2	
4-Nitroquinoline-N-Oxide	PPC-NQ02	50 µg	1	–	1	
9-Aminoacridine	PPC-AR05	1000 µg	1	–	1	2–8°C
N4-Aminocytidine	PPC-AC02	2500 µg	1	–	1	
2-Aminoanthracene	PPC-AA01	100 µg	1	–	3	
2-Aminofluorene	PPC-AF10	10 mg	1	–	1	
S9 100/1537 Booster Solution	PRS-BB01	500 µL	1	–	1	2–8°C
Growth Medium	PMM-GM00	50 mL	2	18	18	18–25°C
Exposure Medium TA Strains	PMM-EM02	50 mL	2	12	12	18–25°C
Indicator Medium TA Strains	PMM-IM10	550 mL	2	12	12	18–25°C
Exposure Medium E.coli Strains	PME-EM22	50 mL	1	3	3	18–25°C
Indicator Medium E.coli Strains	PME-IM31	550 mL	1	3	3	18–25°C

[1]: if no –80°C storage is available at your institution, please contact Xenometrix at info@xenometrix.ch.
[1]: products stored at 18–25°C should be stored in the dark.
[2]: the bacteria are shipped with cool packs. Upon arrival they must be immediately stored at least at –80°C. Improper storage at –20°C may compromise the viability of the strains. The tubes are not suitable for liquid nitrogen storage.
[3]: each vial has 50 µL and should be resuspended in 200 µL of Growth Medium (final volume in the vial: 250 µL).
[4]: by exception PRS-PB02 S9 lyophilized 2 mL x 1 will be packed in place of 1 mL x 2 in B01-513-S2-P and PRS-PB02 S9 lyophilized 1 mL x 20 will be packed in place of 2 mL x 10 in B10-513-S2-P.
[5]: once dissolved, positive controls must be aliquoted and stored at –20°C. Multiple freeze-thaw cycles result in loss of activity. Xenometrix does not take any responsibility for loss of activity of positive controls.

S9 Cofactor Kit (Art. No. PCO-0800)
S9 buffer components are not included in the Ames MPF™ kit. A ready-to-use kit available separately from Xenometrix containing phosphate buffer pH 7.4, MgCl2, KCl, G-6-P and NADP for preparing the S9 mix.

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
- Light table for scoring results (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
- 5–50 µL and 50–200 µL 8-channel pipettes
- 8-Channel repeating pipettor (dispenser) and sterile tips (highly recommended)
- Sterile 50-mL tubes with regular caps or 50-mL tubes with filter caps (or sterile cell culture flasks, small Erlenmeyer)
- Sterile 24-well exposure plates, sterile 384-well microtiter plates and sterile 96-well microtiter plate
- Sterile Reagent reservoirs
- Sterile 5-mL and 10-mL pipettes
- Spectrophotometer cuvettes
- Solvents for sample dilution and solvent control (e.g., DMSO, ddH2O, …)
- Sterile S9 buffer components
- Plastic foil in case compounds are volatile (supplier: ThermoScientific, Art.No.: 236366).

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

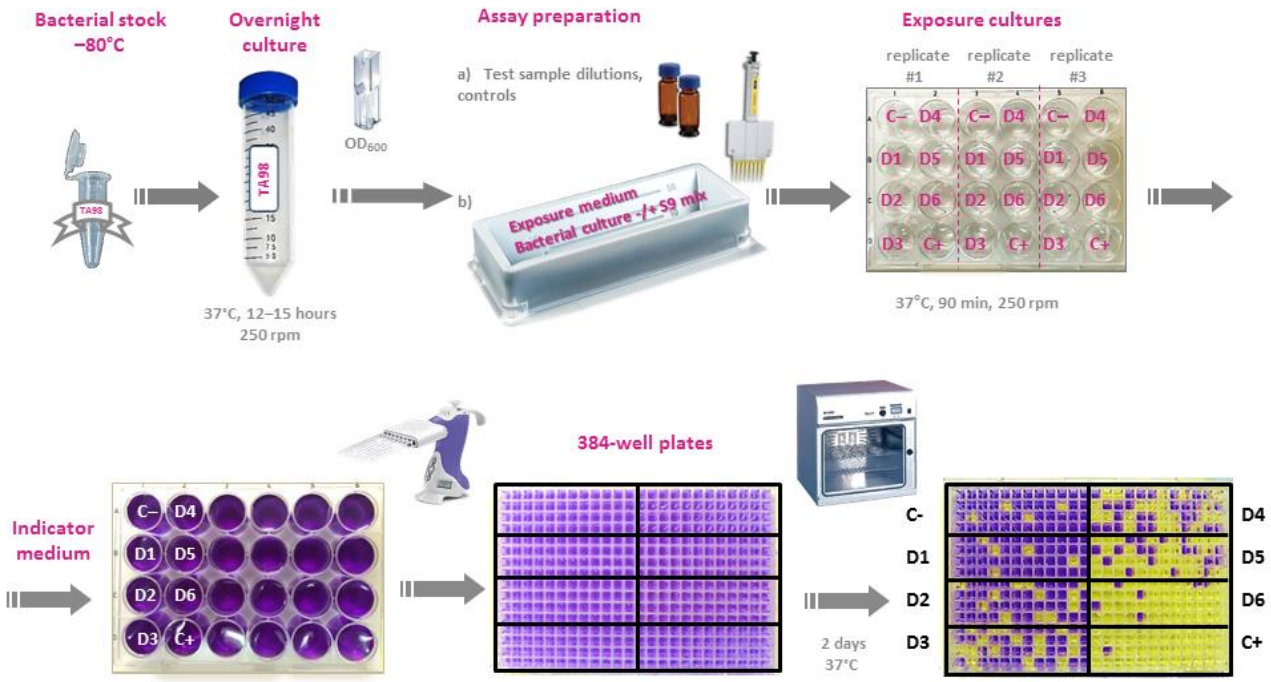
- Please consult your local guidelines for handling *S. typhimurium* strains in the lab. The strains used in this kit are of low pathogenicity and are generally assigned in Risk Group Level 1 depending on country-specific regulations. You may consult [3] for more information.
- All kit components are not for use in humans and animals, 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
Before starting the first tests, we warmly recommend watching the Visual Guide available:

- on the Xenometrix website: https://www.xenometrix.ch/ames-test-scientific-background.html
- on YouTube: https://www.youtube.com/watch?v=-nwsyLBwjaY

Note 8
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.

7. Assay Procedure



8. References

[1] https://www.oecd.org/chemicalsafety/risk-assessment/1948418.pdf

[2] Mortelsmans K, Zeiger E. 2000. The Ames Salmonella/microsome mutagenicity assay. Mutat Res. 455:29–60.

[3] http://www.absa.org/riskgroups/bacteria.html

[4] Heringa MB, Harmsen DJH, Beerendonk EF, Reus AA, Krul CAM, Metz DH, IJpelaar GF. 2011. Formation and removal of genotoxic activity during UV/H2O2-GAC treatment of drinking water. Water Res. 45:366–374.

[5] Piegorsch WW, Simmons SJ, Margolin BH, Zeiger E, Gidrol XM, Gee P. 2000. Statistical modeling and analyses of a base-specific Salmonella mutagenicity assay. Mutat Res. 467:11–1.

Other helpful publications not cited in the manuscript:

[6] Flückiger-Isler S, Kamber M. 2012. Direct comparison of the Ames microplate format (MPF) test in liquid medium with the standard Ames pre-incubation assay on agar plates by use of equivocal to weakly positive test compounds. Mutat Res. 747(1):36–45.

[7] Spiliotopoulos D, Koelbert C. 2020. Assessment of the miniaturized liquid Ames microplate format (MPF™) for a selection of the test items from the recommended list of genotoxic and non-genotoxic chemicals. Mutat Res. 856–857:503218.

[8] Rainer B, Pinter E, Prielinger L, Coppola C, Marin-Kuan M, Schilter B, Apprich S, Tacker M. 2021. Direct comparison of the lowest effect concentrations of mutagenic reference substances in two Ames test formats. Toxics. 9(7):152.

[9] Kamber, Sini Flueckiger-Isler, Guenter Engelhardt, Rudolf Jaeckh, Errol Zeiger, Comparison of the Ames II and traditional Ames test responses with respect to mutagenicity, strain specificities, need for metabolism and correlation with rodent carcinogenicity, Mutagenesis vol. 24 no. 4 pp. 359–366, 2009

[10] C.V. Chandrasekaran *, K. Sundarajan, Kripalini David, Amit Agarwal, Toxicology in Vitro 24 (2010) 885–897 In vitro efficacy and safety of poly-herbal formulations

Please refer to the Xenometrix website for more publications https://www.xenometrix.ch/ames-mpf-31.html