Ames MPF™ Penta 2

Microplate Format Mutagenicity Assay

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

Short Protocol

For Research use only **Version 2.0 May 2018**

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 Email: info@xenometrix.ch

Principle of the Test

Point mutations were made in the histidine (*Salmonella typhimurium*) or the tryptophan (*Escherichia 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. When a mutagenic event occurs, 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, respectively.

A sample's mutagenic potential is assessed by exposing these amino acid-requiring organisms to varying concentrations of chemical 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 / tryptophan prototrophy to survive and grow.

The available strains are the *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537, and the *E. coli* strain wp2 *uvrA*[pKM101]. TA100, TA1535 and the *E. coli* strains are for the detection of base substitution mutations and TA98 and TA1537 are 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 their primary reversion site and detect certain oxidizing mutagens, cross-linking agents and hydrazines.

The available strains meet the requirements of the OECD guideline 471 for testing of chemicals.

Assay Description

Bacteria are exposed to 6 concentrations of a test agent, 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 and aliquoted into 48 wells of a 384-well plate. Within two days, cells that have undergone reversion to amino acid 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.

A dose dependent increase in the number of revertant colonies upon exposure to test sample relative to the solvent control indicates that the sample is mutagenic in the Ames MPF assay.

The mutagenic potential of samples is assessed directly and in the presence of liver S9 fractions.

Changelog:

Date	**New version**	**Changes**
18.05.2018	2.0	• Changelog added • Minor text modifications.

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

Strain	Mutation	Type	Cell Wall	Repair	pKM101
S.typhimurium					
TA98	*hisD3052*	Frameshifts	*rfa*	*uvrB*	yes
TA100	*hisG46*	Base-pair subst.	*rfa*	*uvrB*	yes
TA1535	*hisG46*	Base-pair subst.	*rfa*	*uvrB*	no
TA1537	*hisC3076*	Frameshifts	*rfa*	*uvrB*	no
E. coli WP2					
uvrA[pKM101]	*trpE65*	Base-pair subst.	-	*uvrA*	yes

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 (Mortelsmans and Zeiger (2000), Mutat. Res. 455, 29-60).

uvrA/uvrB: The uvrA/uvrB 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 Ames MPF™ Penta 2 Mutagenicity Assay kit contains the following components and should be stored as indicated:

–70°C:

Vials containing *S. typhimurium* (TA98, TA100, TA1535, TA1537) and *E. coli* strain (wp2 *uvrA*[pKM101])

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
- Dissolved positive controls
- S9-NADP, S9-G-6-P (provided only with the S9 Cofactor kit)

4°C:

- Positive controls before reconstitution
- S9 100/1537 Booster solution (provided only in kits with S9)
- S9-Buffer-Salts (provided only with the S9 Cofactor kit)

20 – 25°C (room temperature, protected from light):

Growth Medium
Exposure Medium
Indicator Medium

Required equipment and consumables NOT included with the kit

Note: all plastic ware has to be sterile!

- Environmental shaker capable of 37°C, 250 rpm incubations with approx. 2.5 – 3 cm amplitude. For incubators with smaller amplitude, alternative incubation vessels and rotational speeds can be used.
- 37°C dry incubator
- Light table for scoring results (recommended)
- Spectrophotometer 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 and sterile tips (highly recommended)

- 50 ml tubes with (filter) caps; alternatively Erlenmeyer flasks
- 24-well plates
- 384-well microplates
- 96-well microplate
- Reagent reservoirs
- 5 ml and 10 ml pipettes
- Spectrophotometer cuvettes
- Solvents for sample dilution and solvent control
- S9 buffer components*****

Included in some kit versions only:

- Positive control chemicals
- S9 liver fraction (Aroclor 1254 or Phenobarbital/β-Naphtoflavone-induced), including S9 100/1537 Booster solution

***S9 Cofactor kit (Art. No. PCO-0800)**
A ready-to-use kit available separately from Xenometrix containing phosphate buffer pH 7.4, $MgCl_2$, KCl, G-6-P and NADP for preparing the S9 mix. This kit replaces the self-made S9 buffer components.

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 *http://www.absa.org/riskgroups/bacteria.html* 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'.

Ames MPF – Assay Procedure