XENOMETRIX

Swiss Commitment for Bioassays

Ames II

Microplate Format Mutagenicity Assay

S. typhimurium TA98 and TAMix

Short Procedure

For Research use only

Version 5.03 May 2018

NOTE 1:

This manual applies to the following versions of the kit:

Article No.	Kit size*	Lyophilized liver S9	Positive Controls[#]
E01-213-S1-P (Aroclor 1254-induced S9)	1	+	2-NF, 4-NQO, 2-AA
E01-213-S2-P (PBN- induced S9[§])	1	+	2-NF, 4-NQO, 2-AA
E10-213	10	-	-
E10-213-S1-P (Aroclor 1254-induced S9)	10	+	2-NF, 4-NQO, 2-AA
E10-213-S2-P (PBN-induced S9[§])	10	+	2-NF, 4-NQO, 2-AA

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

[§] PBN-induced S9: Phenobarbital/β-Naphtoflavone-induced S9

Changelog:

Date	**New version**	**Changes**
15.02.2018	5.02	• Changelog added • Minor text modifications.
23.05.2018	5.03	• Note 1 added • New Assay Procedure graphics.

Principle of the Test

Point mutations were made in the histidine (*His*) 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 *His* gene may cause a reversion to histidine prototrophy. These reverted bacteria will then grow in histidine-deficient media.

A test sample's mutagenic potential is assessed by exposing these *his-* organisms to varying concentrations of sample and selecting for the reversion event. Medium lacking histidine is used for this selection which allows only those cells that have undergone the reversion to histidine prototrophy to survive and grow.

The strains provided in this kit are the *Salmonella typhimurium* TAMix and TA98. The Ames II TAMix contains a mixture of equal proportions of the Ames II TA7001–TA7006 strains. Individually, these strains are designed to revert by only one specific base-pair substitution out of six possible changes. Thus, when mixed, all six base substitution mutations can be represented in one culture. This kit also contains TA98 for the detection of frameshift mutations.

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 to support approximately two cell divisions. After exposure, the cultures are diluted in pH indicator medium lacking histidine, and aliquoted into 48 wells of a 384-well plate. Within two days, cells that have undergone the reversion to histidine 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 controls indicates that the sample is mutagenic in the Ames II assay.

The mutagenic potential of samples is assessed directly and in the presence of metabolic activation.

Genotypes of the TA98 and TAMix *Salmonella typhimurium* strains

Strain	Mutation	Type	Target	Cell Wall	Repair	pKM101
TA98	*hisD3052*	Frameshifts	GCGCGCGC	*rfa*	*uvrB*	yes
TAMix contains:						
TA7001	*hisG1775*	Base-pair subst.	A:T>G:C	*rfa*	*uvrB*	yes
TA7002	*hisC9138*	Base-pair subst.	T:A>A:T	*rfa*	*uvrB*	yes
TA7003	*hisG9074*	Base-pair subst.	T:A>G:C	*rfa*	*uvrB*	yes
TA7004	*hisG9133*	Base-pair subst.	G:C>A:T	*rfa*	*uvrB*	yes
TA7005	*hisG9130*	Base-pair subst.	C:G>A:T	*rfa*	*uvrB*	yes
TA7006	*hisC9070*	Base-pair subst.	C:G>G:C	*rfa*	*uvrB*	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).

uvrB: The 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 II Mutagenicity Assay kit contains the following components and should be stored as indicated:

–70°C:

- Vials containing *Salmonella* strains (TA98, TAMix)

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

Note: The bacteria are shipped on dry ice and must be 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 (if included, see Note 1 at beginning of document for available kit configurations)
- Dissolved positive controls
- S9-NADP, S9-G-6-P (provided only with the S9 Cofactor kit)

4°C:

- Positive controls before reconstitution (if provided, see Note 1 at beginning of document)
- 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 shakers with smaller amplitude, alternative incubation vessels and rotational speeds can be used (see section “Assay procedure day 1” of the Instructions for Use).
- 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
- 24-well plates
- 384-well microtiter plates
- 96-well microtiter plate
- 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 (see Note 1 at beginning of this manual):

- Positive control chemicals: 2-nitrofluorene and 4-nitroquinoline N-oxide (for tests without S9) and 2-aminoanthracene (for tests with S9)
  Liver S9 fraction (Aroclor 1254 or Phenobarbital/β-Naphtoflavone-induced)

***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 (Appendix B).

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 II – Assay Procedure