XENOMETRIX

Swiss Commitment for Bioassays

# umuC Easy

## 1-Day Microplate Format Genotoxicity Assay for testing of concentrated (G06…) or aqueous (F06…) samples

### using *S. typhimurium* TA1535/pSK1002 with media and reagents as described in ISO 13829

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# Short protocol

**6 - Sample Kit (2 x 96 determinations)**

Art. No.: G06 - 118
G06 - 118 - S1 – P
F06 - 118
F06 - 118 - S1 - P

Upon receipt of your umuC Easy Genotoxicity Assay kit, **make sure that all reagents are stored appropriately (see pg. 4 for storage instructions)**. 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 e-mail: info@xenometrix.ch

# umuC Easy
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# Principle of the Test

*Salmonella typhimurium* TA1535 [pSK1002] bacteria are exposed to potentially genotoxic test compounds. If genotoxic lesions are produced by the test compound, the umuC gene is induced as part of the bacterial SOS response. The plasmid pSK1002 contains the umuC gene fused to the lacZ reporter gene. The induction of lacZ is measured by the conversion of colorless ONPG substrate (*o*-nitrophenyl-*β*-D-galactopyranoside) to the yellow product *o*-nitrophenol by the lacZ-encoded *β*-galactosidase.

Since different kinds of genotoxic lesions lead to the induction of the SOS response, one strain of *S. typhimurium* with the appropriate reporter gene construct is sufficient to identify all classes of bacterial genotoxins. As with other bacterial genotoxicity and mutagenicity assays, compounds requiring metabolic activation for activity can be identified by the addition of S9 microsomal rat liver extract.

**The protocol was adapted from ISO 13829 "Water quality - Determination of the genotoxicity of water and waste water using the umu-test". All media and reagents except for the S9 co-factor concentrations are as described in ISO 13829 and can be used with the original ISO 13829 protocol or with this optimized umuC- Easy protocol.**

***For the testing of aqueous samples please use the umuC Easy AQ kit!***

# Assay Description

*S. typhimurium* TA1535 [pSK1002] bacteria in the exponential growth phase are exposed for 120 minutes to 4 concentrations of a test sample, as well as to a positive and a negative control. After 2 hrs, the exposure cultures are diluted in fresh medium and allowed to grow for another 2 hrs. The induction and expression of the umuC - lacZ reporter gene is then assessed after lysis of the bacteria. Colorless ONPG is converted to the yellow product *o*-nitrophenol in the presence of induced *β*-galactosidase (lacZ). The intensity of the color correlates with the amount of *β*-galactosidase present and thus with the genotoxic potency of the test compound.

Measurement of the $OD_{600}$ before and after the 2 hr growth phase allows to calculate an Induction Ratio (IR) and to identify toxic growth inhibitory effects.

The genotoxic potential of substances can be assessed directly or in the presence of liver S9 fractions.

# Genotype of S. typhimurium 1535 [pSK1002]

| Strain | Cell Wall | Repair | pSK1002 |
|---|---|---|---|
| **TA1535** | *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.

*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.

pSK1002: The multicopy plasmid pSK1002, a fusion product of genes of the *umu* operon (which was derived from *E. coli* K12 AB1157) with the vector pMC1403 (a pBR322 derivative), carries a *umu*C'-*lac*Z gene fusion product. The *umu* operon is regulated by the SOS genes *rec*A (protease) and *lex*A (repressor) and consists of a promoter, operator, *umuD* and *umuC* genes. The vector carries the *lacZ* and *lacY* genes, the ampicillin resistance gene and *ori*.

# Safety Precautions

- 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
- TA1535 [pSK1002] is classified as a genetically modified organism. Please consult with your local authorities about required permissions and rules for handling.

# Kit Components

Note1:

Each Xenometrix umuC Easy kit contains enough bacteria, media and reagents for 192 determinations. This allows e.g. to test a total of 6 compounds, 4 dilutions each, in triplicates, with positive and negative controls, in the absence and presence of S9. The assay can be performed as 1 or 2 separate experiments (6 or 3 compounds per experiment, respectively).

Alternative plate layouts, dilution schemes or replicate numbers are possible, but are not described in this manual and are not supported by the Excel calculation sheet provided by Xenometrix.

Note2:

The umuC Easy TG medium corresponds to the TGA medium described in the ISO 13829 protocol, except that ampicillin is added just prior to use.

**Kit contents:**

- 2 vials containing *S. typhimurium* TA1535 [pSK1002] in a semi-solid medium. Each vial contains 50 µl to which 200 µl TG medium has to be added just prior to use.
- 4 vials containing sterile ampicillin
- ONPG substrate solution
- 2-mercaptoethanol
- TG medium
- B-buffer
- Stop reagent
- 1 vial each of positive control chemicals 4-Nitroquinoline-N-oxide (4-NQO) and 2-Aminoanthracene (2-AA) (F/G06-118-S1-P only)
- 2 vials with Aroclor 1254-induced rat liver S9 (F/G06-118-S1-P only)

# Storage Conditions

Each Xenometrix umuC Easy kit is shipped at ambient temperature. Please contact Xenometrix if you received the kit later than 10 days after the shipment date indicated on the delivery note (phone: ++41-61-482-14-34; fax: ++41-61-482-20-72, or e-mail: info@xenometrix.ch).

The shipment contains the following components which should be stored **immediately upon arrival** as follows:

**1. -70°C to -80°C:**

- Vials containing *S. typhimurium* TA1535 [pSK1002] in a semi-solid medium.

➨ The bacteria must be stored at least at -70°C. Improper storage at -20°C may compromise the viability and performance of the strain. The tubes are not suitable for liquid nitrogen storage.

(If no -70°C storage is available at your institution please contact Xenometrix)

**2. -20°C:**

- Ampicillin
- ONPG substrate solution. Protect from light!

- S9 (when included)
- S9 buffer components: Glucose-6-phosphate, NADP (not provided, preparation see next page)
- Dissolved positive controls

**3. 2 - 8°C**

- 2-mercaptoethanol

- S9 buffer components: KCl, $MgCl_2$ (not provided, see next page)
- Solid positive controls (when included)

**4. 20 - 25°C (room temperature, protected from light):**

- TG culture medium
- B-buffer
- Stop reagent

# Required Equipment and Consumables NOT Included with the Kit

Note: all plastic ware has to be sterile!

- Environmental shaker capable of 37°C and 28°C, 150 - 250 rpm incubations
- 37°C dry incubator
- Spectrophotometer for measuring optical density at 600 nm
- Microplate reader capable to read at 420 and 600 nm
- 20 µl and 200 µl adjustable pipettes and sterile tips
- 5-50 µl and 50-200 µl 8-channel pipettes

- 50 ml tubes with (filter) caps
- 96-well microtiter plates
- Reagent reservoirs
- 5 ml and 10 ml pipettes
- Spectrophotometer cuvettes
- Solvents for sample dilution and zero dose control
- S9 (provided only in kits G06-118-S1-P and G06-118-S2-P)
- S9 buffer components and cofactors

**Cofactor Stock solutions for S9:**

| Stock concentration | Reagent | MW | Total volume | Amount | Storage |
|---|---|---|---|---|---|
| 1.00 M | KCl | 74.55 | 50 ml | 3.728 g | 4°C |
| 0.25 M | $MgCl_2$x6 $H_2O$ | 203.31 | 50 ml | 2.541 g | 4°C |
| 0.20 M | Glucose-6-P[a] | 282.10 | 10 ml | 0.564 g | -20°C |
| 0.04 M | NADP[b] | 765.40 | 10 ml | 0.306 g | -20°C |

[a] as Glucose-6-phosphate sodium salt
[b] as NADP sodium salt hydrate, MW of anhydrous form

- filter-sterilize and store as indicated (KCl and $MgCl_2$ can also be autoclaved)