

Rapid Mutagenicity Testing by Xenometrix:

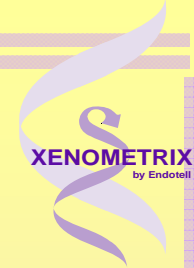
umuC Easy AQ
and
umuC Easy CS



8580 Gove Court • Mason, OH 45040
Phone: 513.770.1991 Toll Free: 866.783.3797 Fax: 513.573.9241 Email: info@aniara.com

www.aniara.com

Form AA36
09-2009



Different kinds of Mutagenicity Testing

Bacterial Assays

„rapid“ SOS Response Assays:

UmuC-Assay
(ISO 13829)

SOS-Chromotest

Vitotox Assay

Ames Mutagenicity Tests:

traditional Ames
(OECD guideline 471)

Ames MPF Penta I
(OECD guideline 471)

Ames MPF (TA98/TA100...)

Ames II

Mammalian Cell Assays

Micronucleus Assay
(OECD guideline draft 487)

Mouse lymphoma TK assay
(OECD guideline 476)

Chromosom Aberration Test
(OECD guideline 473)

Comet Assay

Greenscreen Assay
etc.

Form AA36
09-2009

Different kinds of Mutagenicity Testing

Bacterial Assays

„rapid“ SOS Response Assays:

umuC-Assay
(ISO 13829)

SOS-Chromotest

Vitotox Assay

Ames Mutagenicity Tests:

traditional Ames
(OECD guideline 471)

Ames MPF Penta I
(OECD guideline 471)

Ames MPF (TA98/TA100...)

Ames II

Mammalian Cell Assays

Micronucleus Assay
(OECD guideline draft 487)

Mouse lymphoma TK assay
(OECD guideline 476)

Chromosom Aberration Test
(OECD guideline 473)

Comet Assay

Greenscreen Assay
etc.

Form AA36
09-2009



When do you use which assay?

a) Guideline conform (e.g. endproduct testing):

- Ames plate incorporation test
- Ames MPF Penta I (OECD 471 conform strains)
- Certain mammalian cell tests (e.g. Micronucleus)

b) Screening of compounds:

- Ames MPF (e.g. TA98/TA100), Ames II
- Ames plate incorporation test
- Rapid bacterial tests (using SOS response)

Form AA36
09-2009

When do you use which assay?

a) Guideline conform (e.g. endproduct testing):

- Ames plate incorporation test
- Ames MPF Penta I
- certain mammalian cell tests

b) Screening of compounds:

- Ames MPF, Ames II
- Ames plate incorporation test

-rapid bacterial tests (using SOS response)

-> ca. 4-6h assay time

-> cost efficient

Form AA36
09-2009



umuC Easy Assays by Xenometrix

- Principle:

Uses SOS response in bacteria for indication of DNA damage

DNA damage -> SOS response ↑ -> umuC ↑

Form AA36
09-2009

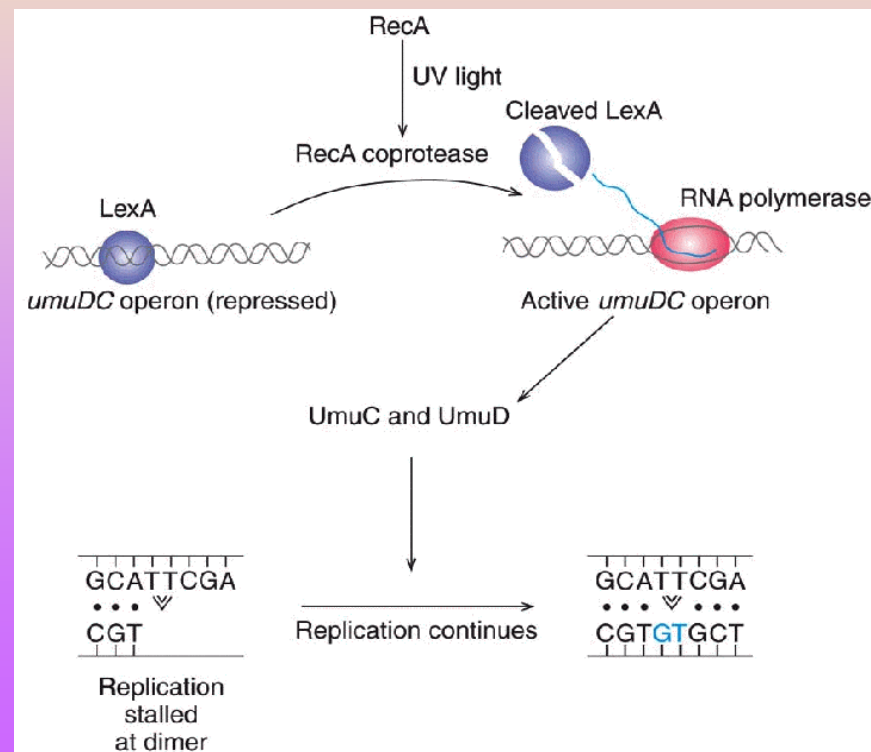
umuC Easy Assays by Xenometrix

-Principle:

Uses SOS response in bacteria for indication of DNA damage

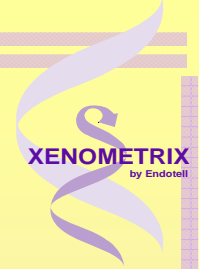
DNA damage -> SOS response ↑ -> umuC ↑

SOS response in bacteria:



Form AA36
09-2009

umuC Easy Assays by Xenometrix



- Strain: ***S. typhimurium* TA1535 (pSK1002)**

pSK1002: umuC-lacZ-fusion in umuC gene
Ampicillin-resistance

TA1535: rfa: permeability for bulky chemicals
uvrB: error-prone DNA repair

- ISO 13829 method

- 1-day microplate format mutagenicity assay (o/n incubation + 6h)

- Also suitable for detecting crosslinking chemical compounds

- 2 x 96 well plates: assay for 6 samples with 4 dilutions, triplicates,
+/- S9, NC, PC

- Two kit variations: umuC Easy AQ (for water samples)
umuC Easy CS (for concentrated samples)

Form AA36
09-2009



Procedure of the umuC Easy AQ and CS assay:

(optimized by Xenometrix)

Day 1: (ca. 5 minutes total time)

- Overnight culture preparation (like with other Ames semi-solid strains)

Day 2: (ca. 6h total time)

- Measure OD₆₀₀ of overnight culture (> 2.0)
- Reincubation with 1:4 dilution (1.5h)
- Exposure with test samples on 96 well plate, 2 h, 37°C
- Dilution step: Second incubation on 2nd plate
- Transfer to 3rd plate, 30min incubation with ONPG substrate
- Stop reagent, measurement of OD₄₂₀ of plate
- Calculation of β -Gal-Induction rate (use Xenometrix Excel data sheet)

Form AA36
09-2009



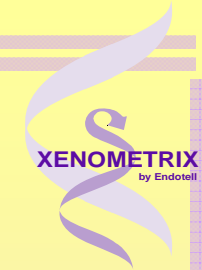
Procedure of the umuC Easy assay:

Minor changes as compared to original ISO 13829:

- Overnight culture preparation: centrifugation and resuspension step
- Different plate layout

-> Customer is free to use original ISO 13829 description with our kit

Form AA36
09-2009



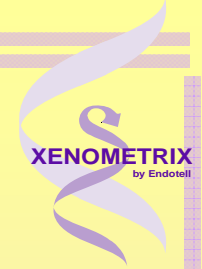
Recommended umuC Easy AQ plate layout if working according to ISO and for optimal pipetting:

Rows A - D without S9 - Rows E - H with S9

	1	2	3	4	5	6	7	8	9	10	11	12			
A	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1 - S3	Samples 1 - 3	
B	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	PC	Positive control	
C	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3	NC	Negative control	
D	PC	PC	PC	SC	SC	SC	NC	NC	NC	BL	BL	BL	SC	Solvent control	
E	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	BL	Blank	
F	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2			
G	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3			
H	PC	PC	PC	SC	SC	SC	NC	NC	NC	BL	BL	BL			
	1.5x			3x			6x			12x			Final sample dilution		

Form AA36
09-2009

Recommended umuC Easy CS plate layout if working according to ISO and for optimal pipetting:



Rows A - D without S9 - Rows E - H with S9

	1	2	3	4	5	6	7	8	9	10	11	12		
A	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1 - S3	Samples 1 - 3
B	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	PC	Positive control
C	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3	NC	Negative control
D	PC	PC	PC	SC	SC	SC	NC	NC	NC	BL	BL	BL	SC	Solvent control
E	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	BL	Blank
F	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2		
G	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3		
H	PC	PC	PC	SC	SC	SC	NC	NC	NC	BL	BL	BL		
	25x			50x			100x			200x			Final sample dilution	

Form AA36
09-2009



Kit Components

- Strain TA1535 (pSK1002) in semi-soli format
- Reagents for two 96well plates: 6 samples , 4 dilutions each, in triplicates
- Positive and negative controls available separately

Storage Conditions

Store at **-70°C**:

- Strain TA1535 (pSK1002) in semisolid format (like Ames MPF)

Store at **-20°C**:

- Ampicillin
- ONPG substrat solution

Not included in the kit:

- S9
- Positive controls

Form AA36
09-2009