The Ames MPF™ 98/100 Assay: Novel Mutagenicity Testing in Liquid Microplate Format using *S. typhimurium* TA98 and TA100

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Introduction:

Genetic toxicity testing has moved towards the earlier stages of drug discovery in order to identify genotoxic liabilities of new compounds in the pipeline. Scaled-down versions of the original Ames plate incorporation test using the *S. typhimurium* strains TA98 (frameshifts) and TA100 (base-pairs) are often used for this purpose. Because in early development many compounds are available in very small quantities, a liquid microplate version with these strains was developed to decrease compound consumption and to increase the throughput of the assay.

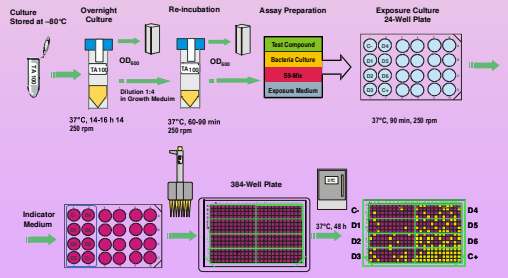
TA98 is already successfully used in the Ames II assay, in combination with TAMix, a mixture of strains to detect base-pair mutations. TA100 with its high spontaneous reversion rate was as yet not suitable for the microplate format with its 48-well upper limit.

We were able to decrease the spontaneous reversion rate of TA100 to a level low enough to be used in the microplate format without loss of sensitivity. The mutagenic responses to 14 reference compounds were compared in TA100 of the Ames MPF and in the TAMix of the Ames II test.

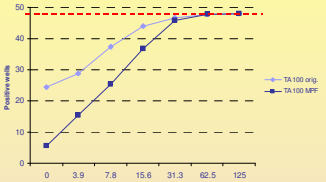
Test method:

The Ames MPF™ assay is performed in 384-well plates with the histidine auxotroph *Salmonella typhimurium* tester strains TA98 (frameshift mutations) and TA100 (base-pair substitutions). After overnight growth, dilution and re-incubation for about 1 hr, exposure with test chemicals is performed in 24-well plates (6 concentrations in triplicate, together with solvent and positive controls) in the absence and presence of S9 mix. After treatment, a specially formulated medium containing a pH indicator and lacking histidine is added. Each well of the 24-well plate is aliquoted into 48 wells of a 384 well-plate and incubated for two days to allow revertant bacteria to form colonies. Mutagenicity (bacterial growth) is measured colorimetrically by a color change (pH drop) from purple to yellow.

The data presented in this poster were done with 4 concentrations.



Results:



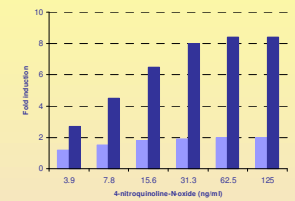


Figure 1: Comparison of the original TA100 strain with TA100 MPF.

Due to its greatly reduced spontaneous reversion rate the „Fold Induction over Zero Dose" is significantly higher and therefore suitable for the microplate format with a upper limit of 48 wells

	TA100 w/o S9	TA100 with S9	TAMix w/o S9	TAMix with S9
Methyl methanesulfonate	2.7 µg/ml		100 µg/ml	
N^4-Aminocytidine	<<12.5 µg/ml		12.5 µg/ml	
Formaldehyde	7.5 µg/ml		7.5 µg/ml	
4-Nitroquinoline-N-oxide	<<7.8 ng/ml	0.5 µg/ml	<<7.8 ng/ml	0.5 µg/ml
Pyrene-1,6-quinone	0.4 µg/ml		2.0 µg/ml	
1,6-Dinitropyrene	<0.004 µg/ml		<0.016 µg/ml	
Cyclophosphamide		<200 µg/ml		1000 µg/ml
Dimethylanthracene	100 µg/ml	20 µg/ml	500 µg/ml	<20 µg/ml
5-Azacytidine	---		25 µg/ml	
2-Aminoanthracene		0.63 µg/ml		0.63 µg/ml
Benzo(a)pyrene		0.016 µg/ml		0.4 µg/ml

not mutagenic -/+ S9: Pyrene, Anthracene, Ethylenediamine

Table 1:

Minimal Mutagenic Dose (MMD) in TA100 and TAMix

MMD was defined as >2-fold induction over baseline, which is the ratio of the mean number of positive wells for the dose concentration divided by the zero dose baseline. The zero dose baseline is obtained by adding one standard deviation to the mean number of positive wells of the zero dose (medium) control.

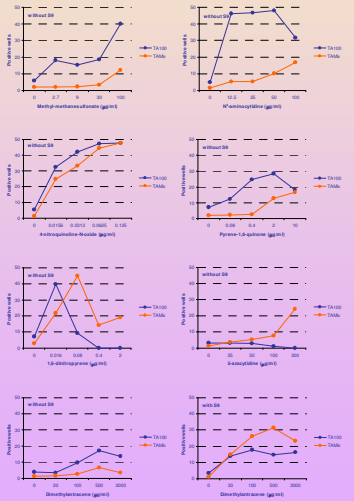


Figure 2: Comparison of TA100 and TAMix (Ames MPF vs. Ames II).

	TA100 MPF w/o S9	TA100 MPF with S9	TAMix w/o S9	TAMix with S9	TA100 plate incorp. w/o S9	TA100 plate incorp. with S9	References TA100 plate incorp.
N^4-Aminocytidine	+++		++		+++		1
Methyl methanesulfonate	+++		+		+++		2
1,6-Dinitropyrene	+++		+++		+++		3
Pyrene-1,6-quinone	++		++				
4-Nitroquinoline-N-oxide	+++	++	+++	+++	+++	+++	4
2-Aminoanthracene	---	+++	---	+++	---	+++	4
Dimethylanthracene	+	++	+	+++	+	++	5
Formaldehyde	++		++		++		6
Cyclophosphamide		+		+		+	5
Benzo(a)pyrene	---	++	---	+++	---	+	4
5-Azacytidine	---		++		?		7
Pyrene	---	---	---	---	---	---	4
Anthracene	---	---	---	---	---	---	5
Ethylenediamine	---	---	---	---	---	---	8

Table 2: Relative mutagenic potential of reference compounds as detected by TA100 MPF, TAMix and TA100 (plate incorporation format)

Scoring: Ames MPF and Ames II: Number of wells with revertant bacteria: +++ 30-48; ++ 15-29; + < 15 > MMD

Ames plate incorporation assay: +++ strong; ++ good; + weak; ? unclear

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3) Kubo, T, Urano, K. and Utsumi, H. (2002) Mutagenicity characteristics of 255 environmental chemicals. J. Health Sci. 48; no. 6; 545-554
4) Hakura A., Shimada H. et al. (2005) Salmonella/human S9 mutagenicity test: a collaborative study with 58 compounds. Mutagenesis 20: no. 3; 217-228.
5) Ashby,B.A. Bridges, D. MacGregor, E. Zeiger. Summary report on the performance of bacterial mutation assays. In: Progress in mutation research Vol. 1. Evaluation of short-term tests for carcinogenesis. Report of the international collaborative program, de Serres, F.J., Ashby,J. (Eds.). Elsevier/North Holland (1981) pp. 49 - 67
6) Donovan, M.R. and Mee, C.D (1993) Formaldehyde is a bacterial mutagen in a range of salmonella and escherichia indicator strains. Mutagenesis 8; no. 6; 577-581
7) Gee, P., Sommers, C.H. et al. (1998) Comparison of base-specific salmonella tester strains with the traditional strains for identifying mutagens: the results of a validation study. Mutation Research 412; 115-130
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Conclusions:

The new Ames MPF™ 98/100 test allows to take advantage of the microplate format while using the same *S. typhimurium* tester strains TA98 and TA100 that are used in the Ames plate incorporation test. The 384-well microtiter format requires about 6x less test compound and consumables, and considerable less hands-on time.

The comparison between TAMix and TA100 in the Ames II and Ames MPF microtiter format gave the following results: Seven compounds were more sensitive in TA100 than in TAMix, and both strains showed similar responses with three compounds. One compound was detected by TAMix only, and three chemicals showed no mutagenic activity in both strains.

A comparison of these compounds between TAMix and TA100 in the microplate format with published data of TA100 in the plate incorporation assay shows an excellent correlation.

The Ames MPF assay is therefore a rapid time- and recource-effective pre-registration alternative to the Ames plate incorporation assay using the same TA98 and TA100 strains of *S. typhimurium*.

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