



Enhanced Ames Test (EAT)

Under standard Ames test conditions, there are concerns about the low detection sensitivity of some N-nitrosamines, such as NDMA.

(For example, the results of carcinogenicity tests of nitrosamines in rodents and the Ames test were inconsistent [Rao et al., 1979]) Enhanced Ames Test (EAT) has been developed to detect the mutagenicity of nitrosamines with greater sensitivity.

Test method

Test conditions	Conventional Ames test	EAT
Strain	TA100, TA1535, TA98, TA1537, WP2uvrA	TA100, TA1535, TA98, TA1537, WP2uvrA (pKN101)
S9	Rat S9	Rat S9, Hamster S9
S9 mix concentration	10%	30%
Test method	Pre-incubation method Plate method, etc.	Pre-incubation method recommended
Pre-incubation time	37°C, 20 minutes	37°C, half an hour

Evaluation method: In accordance with European Medicines Agency (EMA).

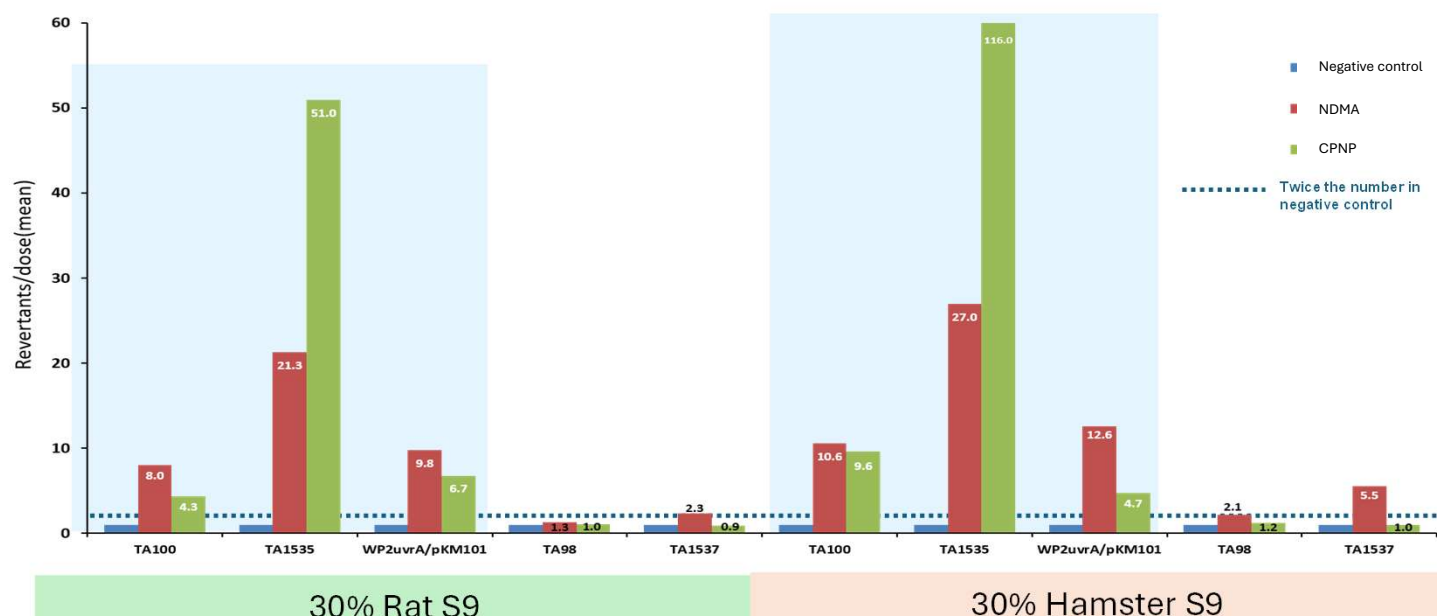
S9: 30% S9 mix is prepared.

Rat S9 (manufacturer: Oriental Yeast Co., Ltd.)/Hamster S9 (manufacturer: MOLTOX)

Positive control: N-nitrosodimethylamine (NDMA)/1-cyclopentyl-4-nitrosopiperazine (CPNP)

Judgment criteria: When the number of colonies is more than twice the number of colonies in the negative control, the results are judged as positive (mutagenic).

Accumulated data of the positive control group (average)



Results

Marked mutagenicity (positive reaction) was observed in TA100, TA1535, WP2uvrA/pKM101.
No positive reaction was noted in part of TA98 and TA1537.

References

- Kajavadara et al., 2024, Selection of solvent and positive control concentration for enhanced Ames test conditions for N-nitrosamine compounds, Regulatory Toxicology and Pharmacology Volume 154, December 2024, 105711, 10.1016/j.yrtph.2024.105711
- Thomas et al., 2025, Resolution of historically discordant Ames test negative/rodent carcinogenicity positive N-nitrosamines using a sensitive, OECD-aligned design, Mutagenesis, Volume 40, Issue 2, April 2025, Pages 116–125, 10.1093/mutage/geae027