Moltox S9 (Post-Mitochondrial Supernatant)

Intended Use: S9 is an exogenous mammalian source of cytochrome-P450 enzymes. When delivered to the test system in the presence of NADP and cofactors for NADPH-supported oxidation (i.e. S9 mix), P450-mediated metabolism of potential carcinogens may result in the generation of metabolites that exhibit activities that are not observed in the parental material.

Warnings and Precautions: For Laboratory Use Only

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, dispose of all materials according to location- specific biohazard procedures.

Storage: Upon receipt, store frozen S9 under ultralow freezer conditions (- 80°C < -70°C). Store lyophilized S9 under standard freezer conditions (approximately – 20°C). When thawing S9, do so under strict observation, preferably thawing S9 on ice. Refreezing of S9 will affect the quality of the material.

Reconstituting lyophilized S9: When reconstituting lyophilized S9, use ice-cold, sterile deionized water. Add a volume of water equivalent to the fill size in milliliters on the product vial. Store the reconstituted S9 on ice until use.

Procedure: As S9 is used in several types of assays, there is no specific method that applies to all testing. Please refer to the following guidelines for information on how to use S9:

***In vitro* genotoxicity tests –**

OECD 471 - Genetic Toxicology: *Salmonella typhimurium*, Reverse Mutation Assay OECD 473 - Genetic Toxicology: *In vitro* Mammalian Cytogenetic Test.
OECD 476 - Genetic Toxicology: *In vitro* Mammalian Cell Gene Mutation Test.
OECD 479 - Genetic Toxicology: *In vitro* Sister Chromatid Exchange Assay in Mammalian Cells.
OECD 480 - Genetic Toxicology: *Saccharomyces cerevisiae,* Gene Mutation Assay.
OECD 481 - Genetic Toxicology: *Saccharomyces cerevisiae,* Mitotic Recombination Assay.
OECD 482 - Genetic Toxicology: DNA Damage and Repair/Unscheduled DNA Synthesis in Mammalian Cells *In vitro.*

OECD guidelines can be obtained free of charge from www.oecd.org.

For additional information on the Reverse Mutation Assay (ie. Ames Assay), refer to "The Ames *Salmonella*/ microsome mutagenicity assay", *Mutation Research*, 2000, Vol 455, p 29 – 60.

S9 requires a NADPH regenerating system, not included. Refer to appropriate test method for information on the appropriate system. Moltox offers a prepared NADPH regenerating system (Regensys A and Regensys B) that may be appropriate for your assay. Please contact Moltox for further information.

Expected Results:

Refer to the appropriate OECD guideline for expected assay results.

Moltox Lyophilized Culture Discs (71– and 72– Part numbers)

Intended Use: For use in the *in vitro* Bacterial Reverse Mutation Test; i.e. Ames Assay. See below table to determine the reversion event that each strain detects and for plasmid information.

Warnings and Precautions:

For Laboratory Use Only

The bacterial strains contained in the lyophilized discs are potential etiologic agents and are intended for use only by those skilled in the safe handling of potentially infectious agents. The strains are considered BioSafety Level 2 organisms and should be handled accordingly [see CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, HHS Publication (CDC) 93-8395. Available from US Government Printing Office, Superintendent of Documents, Washington DC 20402 (Stock No. 017-040-00523-7)].

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. Dispose of cultures according to your institutional biohazard program

Storage: Upon receipt, store discs under refrigeration at 2 – 8°C.

Procedure: Warm the product vial to room temperature and remove the cap and stopper so as to avoid contaminating the assembly. Using a flamed or disposable bacteriological loop, needle, or tweezers, aseptically remove one or more discs and use to inoculate a quantity of Oxoid #2 nutrient broth. Use a vessel that is 3 – 5 times the volume of the culture to ensure adequate aeration. For pKM101-containing strains, add ampicillin to achieve a final concentration of 25 µg/ml. For pAQ1 strains, add tetracycline to achieve a final concentration of 2 µg/ml. Incubate on a shaker (150 - 160 rpm) @ 37°C until 1 – 2 x 10^9 colony forming units/ml is achieved.

S. typhimurium and *E. coli* WP2 strains

Moltox Part Number	Strain Designation	Reversion Event	Plasmid
71-1535L	TA1535	Base-pair substitution	N/A
71-1537L	TA1537	Frameshift	N/A
71-1538L	TA1538	Frameshift	N/A
71-100L	TA100	Base-pair substitution	pKM101
71-097L	TA97a	Frameshift	pKM101
71-098L	TA98	Frameshift	pKM101
71-102L	TA102	Transition/transversion	pKM101, pAQ1
72-187L	WP2	Base-pair substitution	N/A
72-188L	WP2, uvrA	Base-pair substitution	N/A
72-002L	WP2, pKM101	Base-pair substitution	N/A
72-003L	WP2, uvrA, pKM101	Base-pair substitution	N/A

Expected Results:

Refer to the lot specific Quality Control statement and laboratory historical data or SOP for spontaneous and positive control treatment induced reversion rates.