MOLTOX®
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Product Information

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.

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. Thaw only what you expect to use that day; discard any unused S9. Do not refreeze as freeze/thaw cycles of S9 will affect the quality of the material and may cause erroneous results.

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.

NADPH regenerating system: S9 requires an NADPH regenerating system, not included. S9 combined with an NADPH regenerating system is referred to as an S9 mix. Refer to appropriate test method for information on the appropriate system. MOLTOX® offers a prepared NADPH regenerating system (Regensys™ A and Regensys™ B) that, when mixed with S9, may be appropriate for your assay. MOLTOX® also offers a lyophilized complete S9 mix (Mutazyme™) that, when reconstituted, may also be appropriate for your assay.
Please contact MOLTOX® for further information.

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: Bacterial Reverse Mutation Assay

OECD 473 - Genetic Toxicology: *In vitro* Mammalian Cytogenetic Test

OECD 476 - Genetic Toxicology: *In vitro* Mammalian Cell Gene Mutation Tests using the *Hprt* and *xprt* genes

OECD 487 - Genetic Toxicology: *In vitro* Mammalian Cell Micronucleus Test

OECD 490 – Genetic Toxicology: *In vitro* Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene

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

Expected Results: Refer to the appropriate OECD guideline for expected assay results.

Induction Information: PB/BNF – Phenobarbital/β-Napthoflavone

Matushima, et. al. 1976. In: de Serres, F.J, et. al., editors. *In Vitro Metabolic Activation in Mutagenesis Testing*. Amsterdam (NL) Elsevier/North Holland p. 85-88.

Additional Information: For additional information on the Bacterial Reverse Mutation Assay (i.e., the Ames Assay), refer to:

Maron, D. and B.N. Ames (1983) Revised methods for the Salmonella mutagenicity test, Mutat. Res., 133, 173 - 215.

Mortelmans, K. and E. Zeiger (2000) The Ames *Salmonella*/ microsome mutagenicity assay, Mutat. Res., 455, 29 - 60.