## **S9** Certificate of Analysis – Specimen

## **Product Information**

Product:	Phenobarbital/beta-Naphtoflavone induced rat liver S9			
	Postmitochondrial Supernatant, lyophilized			
Article Numbers:	PRS-PB01, PRS-PB02			
Lot:	XXXXXX			
Expiry Date:	XX.XXXX			
Storage:	–20°C or ≤ –70°C			
Tissue/Lyophilization Buffer:	Liver / 0.05 M Tris HCl pH 7.4			
Protein Concentration:	30.2 mg/ml 1			
Country of Origin:	Switzerland			

## **Quality Control Results**

Positive Controls' Dose Responses			Aver	Average Number of Revertants/48 wells			
	2-Aminoanthracene concentration (PPC-AA01, μg/ml)						
Strain	0	0.25	0.5	1.0	2.0	Acceptance	
TA98 <sup>2</sup>	3.0	47.7	48.0	48.0	48.0	Passed	
	2-Aminoanthracene concentration (PPC-AA01, µg/ml)						
Strain	0	0.625	1.25	2.5	5.0	Acceptance	
TA100 <sup>2,3</sup>	4.0	48.0	48.0	48.0	48.0	Passed	
TA1535 <sup>2</sup>	0.3	39.0	43.3	44.3	44.3	Passed	
TA1537 <sup>2,3</sup>	1.0	14.7	24.7	31.7	31.0	Passed	
TAMix <sup>4</sup>	0.8	29.3	44.3	47.3	47.7	Passed	
	2-Aminofluorene concentration (PPC-AF10, µg/ml)						
Strain	0	50	100	200	400	Acceptance	
EC Combo2	6.0	20.7	24.3	24.7	27.0	Passed	
<i>E. coli</i> WP2 uvrA[pKM101] <sup>2</sup>	4.0	22.3	22.7	27.7	33.3	Passed	
	benzo[a]pyrene concentration (µg/ml)						
Strain	0	1.25	2.5	5.0	10.0	Acceptance	
TA98 <sup>2</sup>	1.0	11.7	12.0	20.3	28.3	Passed	
Sterility <sup>a</sup>						Passed	

<sup>a</sup>: suitable for Ames MPF™/Ames II/Ames Agar Plate test, not tested for other applications.

Certificate with MicroAmes6, MacroAmes1 or Hamster S9 available

# **S9 Products available:**

Art.Number	Product Name	Volume
PRS-PB01	Phenobarbital/ -Naphtoflavone induced lyophilized rat liver S9	1 ml
PRS-PB02	Phenobarbital/ -Naphtoflavone induced lyophilized rat liver S9	2 ml
PRS-H-PB05LY	Phenobarbital/ -Naphtoflavone induced lyophilized hamster liver S9	5 ml
PRS-BB01	S9-100/1537 Booster Solution to reduce S9 toxicity in TA100, TA1537	0.5 ml
PCO-0800	S9 Cofactor Kit (S9-Buffer-Salts, S9-G-6-P, S9-NADP)	25 ml

Contact us at:

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# **Post-mitochondrial S9 Fraction from Rat** or Hamster Liver

- mixed).



 Phenobarbital/ beta-Naphtol induced rat or hamster liver S9

 Carefully lyophilized with competence resulting in a high quality S9 product

• Quality controlled in 7 Ames Tester Strains: TA98, TA100, TA1535, TA1537, TAMix, E.coli WP2 uvrApKM[101], E.coli Combo (uvrA and pKM[101]

## Rat liver S9 Performance in the Ames MPF<sup>™</sup> Assay: Aroclor1254 vs Phenobarbital/Naphtoflavone induced S9

#### Dimitrios Spiliotopoulos<sup>1</sup>, Cécile Koelbert<sup>1</sup>

<sup>1</sup>Xenometrix AG, Gewerbestrasse 25, 4123 Allschwil, Switzerland

#### Introduction

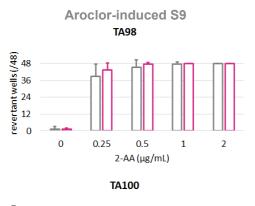
chemicals (e.g., aromatic amines or polycyclic aromatic hydrocarbons) are biologically inactive but become mutagenic upon metabolization, often mediated by the cytochrome-based P450 metabolic oxidation system. This system is present in humans and lower animals (mainly in the liver) but absent in bacteria. An exogenous mammalian organ activation system must therefore be included to the Ames test and, for the most part, the metabolic system is taken from rodents.<sup>(1)</sup> S9 is manufactured from rat or hamster livers which have been treated with substances causing a strong induction of many xenobiotic metabolizing enzymes. Historically, such substance has often been **Aroclor 1254**, but  $\beta$ -naphthoflavone and phenobarbital has also been used.<sup>[2,3]</sup> A homogenate of the liver is subsequently centrifuged. The resulting supernatant, generally referred to as **S9**, contains microsomes and cytosol, and therefore all microsomal and cytosolic xenobiotic metabolizing enzymes.<sup>[3]</sup> Other cofactors, including glucose-6-phosphate (G-6-P) and  $\beta$ -nicotinamide adenine dinucleotide phosphate (NADP, for the NADPH-supported oxidation), are added to the system.<sup>[1]</sup>

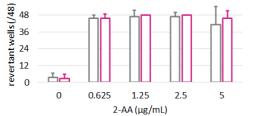
As other commercial polychlorinated biphenyls mixtures, Aroclors have been banned in 1977: these mixtures proved to be toxic to humans (with high bioaccumulation in adipose tissue) and have long environmental persistence.<sup>[4]</sup> As a result, the lots of Aroclor-induced S9 rat liver homogenate were destined to be depleted. Xenometrix AG, in an effort to provide the best products to the Ames II and Ames MPF<sup>III</sup> users, has generated a database of results over the last decade to compare the

performance of the Aroclor 1254-induced S9 and the β-naphthoflavone/phenobarbital-induced S9 with well known and studied mutagens requiring metabolic activation, 2aminoanthracene (2-AA), 2-aminofluorene (2-AF) and benzo[a]pyrene (B[a]P).

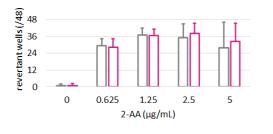
### Performance of the Aroclor 1254- and PB/NF-induced S9 in the Ames II and Ames MPF™: Historical Data

The average and standard deviation values for dose responses for Aroclor 1254- and PB/NF-induced S9 are shown in grey and pink, respectively.

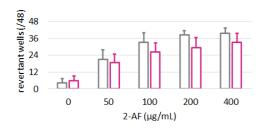


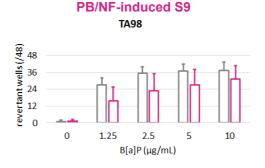




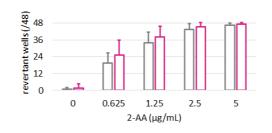




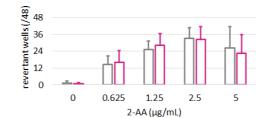




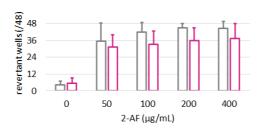
TAMix







E. coli WP2 uvrA[pKM101]



Conclusions

These results suggest that data generated with Aroclor 1254- and PB/NF-induced S9 are essentially identical. They may vary depending on chemical classes.

## Rat liver S9 Performance in the Ames MPF<sup>™</sup> assay: Lyophilized vs Frozen S9

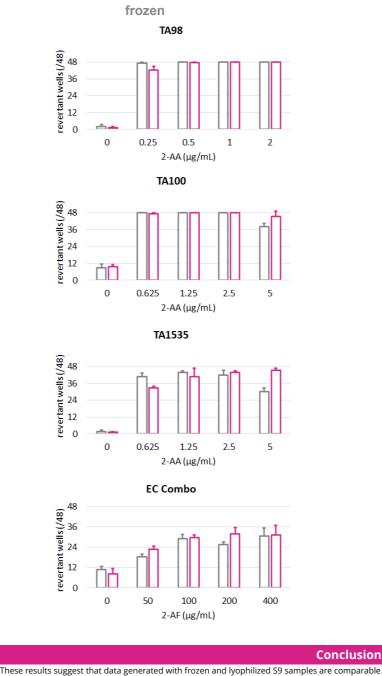
#### Dimitrios Spiliotopoulos<sup>1</sup>, Cécile Koelbert<sup>1</sup>

<sup>1</sup>Xenometrix AG, Gewerbestrasse 25, 4123 Allschwil, Switzerland

Some chemicals (e.g., ) are biologically inactive but become upon metabolization, often mediated by the cytochrome-based P450 metabolic oxidation system in liver. This system is present in humans and lower animals but absent in bacteria. An exogenous mammalian organ activation system must therefore be included to the Ames test and, for the most part, the metabolic system is taken from rodents. $\oplus$ S9 is manufactured from rat or hamster livers which have been treated with substances causing a strong induction of many xenobiotic metabolizing enzymes. Aroclor 1254 and β-naphthoflavone and phenobarbital have been used for this purpose.<sup>[2,3]</sup> A homogenate of the liver is subsequently centrifuged. The supernatant resulting from this centrifugation step, generally referred to as 59, contains microsomes and cytosol, and therefore all microsomal and cytosolic xenobiotic metabolizing enzymes.<sup>[3]</sup> Other cofactors, including glucose-6-phosphate (G-6-P) and β-nicotinamide adenine dinucleotide phosphate (NADP, for the NADPH-supported oxidation), are added to the system separately.<sup>[1]</sup> Freshly prepared S9 of Xenometrix AG is carefully lyophilized by a highly competent partner resulting in a high quality S9 product.

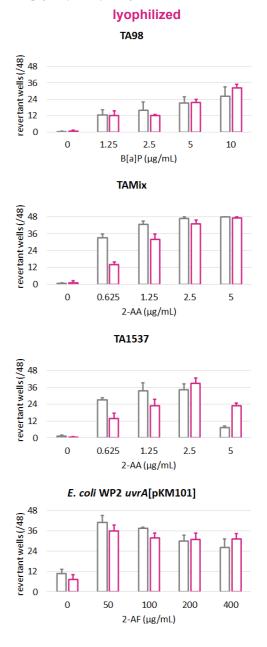
A study suggested that the lyophilized enzymes were generally less stable and less effective than directly frozen preparations, [4] but subsequent efforts to freeze-dry S9 fractions for its long-term storage at ambient temperatures have been reported with more comforting results.<sup>[5,5]</sup> Here, we present a direct comparison of the Xenometrix PB-induced lyophilized S9 and its frozen equivalent for **2-aminoanthracene** (2-AA), **2-aminofluorene** (2-AF) and benzo[a]pyrene (B[a]P).

The average and standard deviation values for dose responses for frozen and lyophilized S9 are shown in grey and pink, respectively.



#### Introduction

Performance of the frozen and lyophilized S9 in the Ames II and Ames MPF™



Conclusions