S9 Performance in the Ames II and Ames MPF™: Lyophilized *Vs.* Frozen

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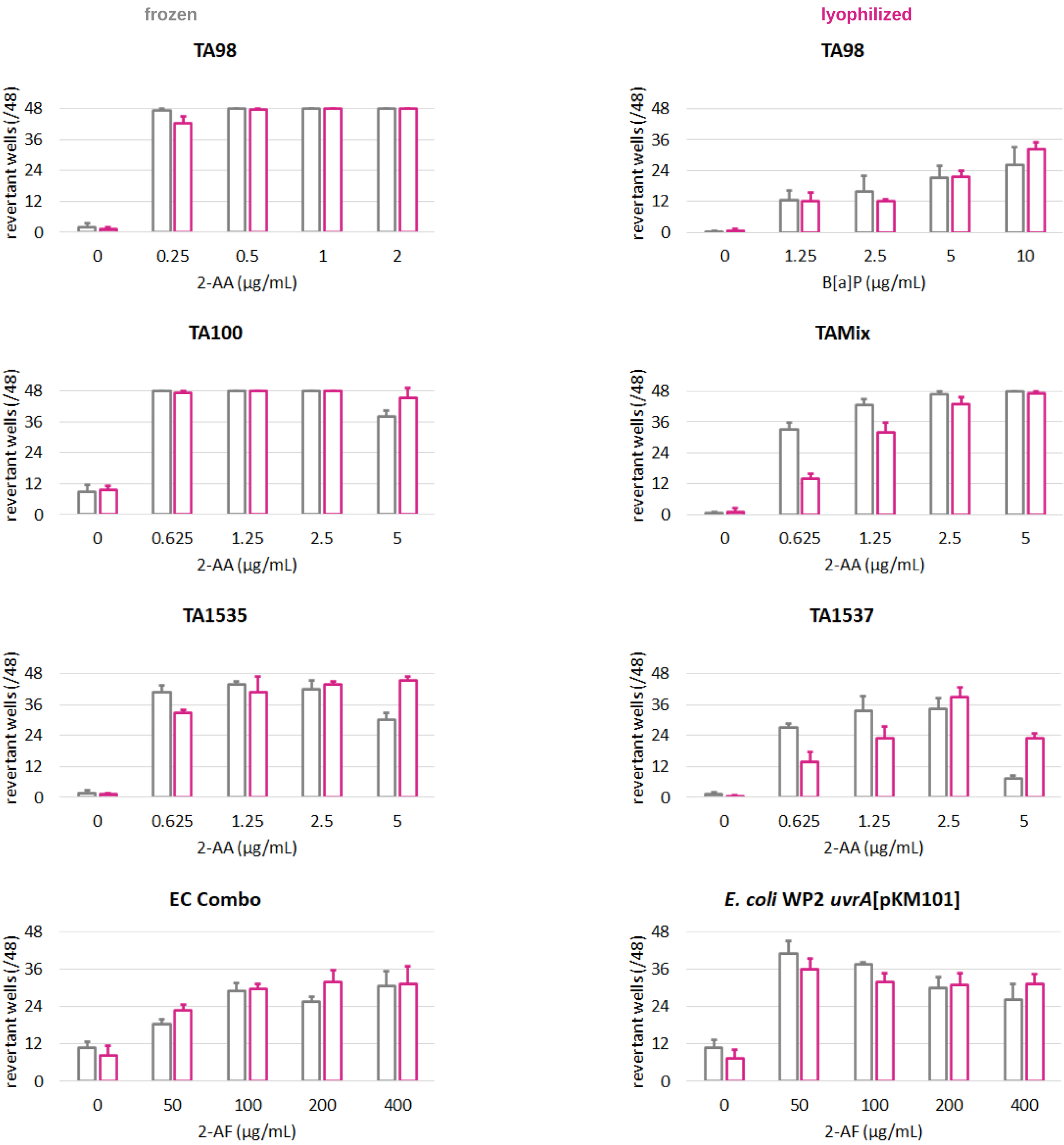
Introduction

Some 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.[1]
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 **β-naphthoflavone and phenobarbital** has also been used.[2,3]
A homogenate of the liver is subsequently centrifuged at 9000 g. The supernatant resulting from this centrifugation step, generally referred to as **S9**, contains microsomes and cytosol, and therefore all microsomal and cytosolic xenobiotic metabolizing enzymes.[3] 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.[1]
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.[5,6]
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).

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

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



Conclusions

These results suggest that data generated with frozen and lyophilized S9 samples are comparable.

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