Ames MPF™ PCO Kit

Microplate Format Mutagenicity Assay

Instructions for use

For additional questions or products, do not hesitate to contact

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For Research use only **Version 4.0, October 2018**

NOTE 1:

This manual applies to the following kit

Article No.	Kit requirements
PCO-0800	1 kit is sufficient for all 1 sample kits, all 10 sample 1- and 2- strain kits 2 Cofactor kits are needed for the 10 sample PENTA 1/PENTA 2 kits.

NOTE 2:

Please refer to the S9 Certificate of Analysis.

The response of *E. coli* to 2-AA is generally lower than that of the *Salmonella* strains, and acceptable values may be difficult to obtain with certain S9 batches.
Xenometrix suggests 2-Aminofluorene (2-AF) as positive control for the *E. coli* strains in the presence of S9. 2-AF is included in the kits containing the *E. coli* strains.

Changelog:

Date	New version	Changes
23.05.2018	1.0	• First version.
01.10.2018	2.0	• Removed references to discontinued products PRS-AC00, PRS-PB00. • Indications on the possibility to freeze and thaw once the dissolved S9 homogenate.
04.10.2018	3.0	• Correction of the “Kit requirements” and Note 2 sections. • Removal of the Table of Contents. • Modifications to the Notes. • Deletion of the paragraphs "Stock Solutions for Preparing 30% S9 mix" and "Preparation of 30% S9 mix using self-made reagents (for 1 test compound)".
18.10.2018	4.0	• Re-formatting of the text. • Minor wording changes.

Assay Description

When dissolved in the suitable solution (cf., PCO kit, PCO-0800), the rat liver S9 homogenate (PRS-AC01/2, PRS-PB01/2, PRS-BB01) provides metabolic activation, which is required by some samples to become mutagenic.

Kit Components and Storage Conditions

Each Xenometrix Ames MPF™ PCO kit contains the following components and should be stored as indicated:

–20°C	S9-Buffer-Salts
4°C	S9-NADP
	S9-G-6-P

Required equipment and consumables NOT included with the kit

Note: all plastic ware has to be sterile!

- 20 µl, 200 µl, and 1000 µl adjustable pipettes and sterile tips
- 5, 15, or 50 ml tubes with caps*
- 5 ml and 10 ml pipettes*

Preparation of 30% S9-Mix

Note: We provide a "S9 100/1537 Booster Solution" to help protecting strains TA100 and TA1537 from possible toxic S9 effects. This solution will be mixed with the Exposure Medium when using S9 in the Ames MPF assay. The booster solution can be used for all *Salmonella* strains to simplify Exposure Medium handling.

The Booster Solution should not be used with *E. coli* strains which are tested in a different Exposure Medium.

Procedure for assays with S9:

Mix the S9 100/1537 Booster Solution with the Ames MPF Salmonella Exposure Medium at a ratio **1 : 667** (*e. g.*, 10 ml Exposure Medium + 15 µl Booster Solution). Prepare the required volume of Exposure Medium / S9 100/1537 Booster Solution mixture.

Note: Once dissolved, the S9 homogenate can be stored at –80°C and thawed once to be used. On the other hand, the S9 Mix must be prepared fresh just before each use.

Preparation of 30% S9 mix using the S9 Cofactor Kit PCO-0800

Keep all (thawed) reagents on ice.
Shortly before use, prepare a 30% S9 mix by combining the volumes of reagents listed below in a sterile tube:

Solution	Volumes for 1 strain	Volumes for 2 strains*	Volumes for 3 strains*	Volumes for 4 strains*	Volumes for 5 strains*
S9-Buffer-Salts	0.748 ml	1.323 ml	1.898 ml	2.531 ml	3.106 ml
S9-G-6-P	0.033 ml	0.058 ml	0.083 ml	0.111 ml	0.136 ml
S9-NADP	0.130 ml	0.230 ml	0.330 ml	0.440 ml	0.540 ml
S9 Fraction	0.390 ml	0.690 ml	0.990 ml	1.320 ml	1.620 ml
Final Volume =	1.301 ml	2.301 ml	3.301 ml	4.402 ml	5.402 ml

*: "Volume for 2 strains": 1 compound on 2 strains or 2 compounds on 1 strain; "Volume for 3 strains": 1 compound on 3 strains or 3 compounds on 1 strain; …

The final concentration of S9 in the culture is 4.5%

Note: Some test results have to be confirmed by repetition (OECD guideline 471), This can be done by modifying the metabolic activation system. For the preparation of 10% or 5% S9 mix, reduce the S9 volume accordingly and add a corresponding volume of sterile water.

Note: Adding the "Booster Solution" to batches of S9 that do not actually need it has no negative effects on the performance of S9 with any of the 4 Ames MPF *Salmonella* strains.

Safety Precautions

- Not for use in humans and animals. For research purposes only.
- Do not drink, eat, smoke, or apply cosmetics in designated work areas. Wear laboratory coats and gloves when handling specimens and kit reagents. Wash hands thoroughly afterwards. Do not pipette by mouth.
- Handle specimens as if capable of transmitting infectious agents. Thoroughly clean and disinfect all materials and surfaces that have been in contact with specimens. Discard all waste associated with specimens in a biohazard waste container.
- Positive control chemicals - although provided in small quantities - are mutagens/carcinogens. Please refer to the corresponding MSDS.

Shipment

If components are damaged or if any problem occurs, please contact Xenometrix by phone +41-61-482-14-34; fax: +41-61-482-20-72; or email: info@xenometrix.ch.