PM Procedures for Streptomyces

SECTION I: MATERIALS

Section A. List of Equipment, Chemicals and Materials

Table 1: Equipment

Equipment	Source	Catalog #
OmniLog PM System	Biolog	93171, 93182, 93184
Turbidimeter	Biolog	3531, 3532, 3585
Multichannel Pipetter	Biolog	3501A, 3505A and B

Table 2: Chemicals and Materials for Inoculation Procedure

Chemicals and Materials	Source	Catalog #
PM panels (PM 1 – 20)	Biolog	12111,12112,12121, 12131,12141,12181, 12182,12183,12161, 12162, 12211-12220
IF-0a GN/GP Base inoculating fluid (1.2x)	Biolog	72268
IF-10b GN/GP Base inoculating fluid (1.2x)	Biolog	72266
Biolog Redox Dye Mix D (100x)	Biolog	74224
Biolog Redox Dye Mix G (100x)	Biolog	74227
sterile cotton swabs	Biolog	3021
sterile pipet tips	Biolog	3001
sterile reservoirs	Biolog	3002
sterile 20 x 150 test tubes	VWR	47729-584
4 oz. (120 mL) Flip-top plastic vial	Capitol Vial Corp.	04CL
3 oz. (90 mL) Flip-top plastic vial	Capitol Vial Corp.	03CL
2 oz. (45 mL) Flip-top plastic vial	Capitol Vial Corp.	02CL

Section B. Additions to Inoculating Fluids

Table 3: Stock Solutions Added to PM Inoculating Fluids

Chemical Stock Solutions	Concen-tration	Formula Weight	Grams/ 100mL	Concen-tration Factor	Sterilized by	Storage Temp
Glucose	500 mM	180.2	9.0	100x	Filtration	RT
Metal ion cocktail						
ZnCl2 7H20 FeCl2 6H2O MnCl2 4H2O CaCl2 2H2O	5.0 mM each	136.3 270.3 197.9 147	68 mg 135 mg 99 mg 74 mg	100x	Filtration	RT

Table 4: Ingredients and Final Concentrations in PM Inoculating Fluids with the Cell Suspensions

Ingredient	PM 1 & 2 Carbon	PM 3, 6, 7, 8 Nitrogen, Phosphorus, Sulfur, Nutrients	PM 9+ Chem Sensitivity
IF-0a	+	+	-
IF-10b	-	-	+
Dye Mix	+	+	+
Glucose	-	5 mM	5 mM
Metal ion cocktail	50 µM	50 µM	50 µM
Cell density	80%T, 1:10	80%T, 1:10	80%T, 1:10

SECTION II: Procedures for PM 1 – 20 Inoculation

Step 1: Prepare a Uniform Cell Suspension

1. Grow the bacterium on a suitable agar medium by streaking for isolated colonies and allow it to grow overnight at 30 ^{o} C. Subculture a second time
2. Remove cells from the agar plate using a sterile swab and transfer into a sterile capped tube containing 25 ml of IF-0. Stir the cell suspension with the swab or do whatever is needed to obtain a uniform suspension. Avoid excessive turbulence which can introduce bubbles.
3. Check the turbidity of the suspension; add cells to achieve 80% T (transmittance).

Step 2a: Inoculation Protocol

1. Label 3 sterile containers for the 3 categories of PM MicroPlates listed below.
2. Fill the sterile containers aseptically according to Table 5 below.

Table 5. Recipe for Inoculating Fluids

Ingredient	PM 1 & 2 Carbon	PM 3 - 8 Nitrogen, Phosphorus, Sulfur, Nutrients	PM 9+
IF-0a	20 mL	60 mL	-
IF-10b	-	-	120 mL
Dye Mix D,G (100X)*	0.24 mL	0.72 mL	1.44 mL
Glucose	-	0.72 mL	1.44 mL
Metal ion cocktail	0.24 mL	0.72 mL	1.44 mL
Water	1.20 mL	2.88 mL	5.76 mL
Cell Suspension	2.32 mL	6.96 mL	13.92 mL
Total	24 mL	72 mL	144 mL

* Try Dye D first. Use Dye D for strongly reducing species and Dye G for weakly reducing species.

Step 2b: Inoculation Protocol

1. Inoculate PM 1,2
   a. Add 2.32 ml of cell suspension to 21.68 ml of PM1,2 inoculating fluid.
   b. Inoculate PM 1 and PM 2 with this cell suspension, 100 ul / well.
2. For PM 3 - 8
   a. Add 6.96 ml of cell suspension to 65.04 ml of PM3 - 8 inoculating fluid.
   b. Inoculate PM 3 - 8 with this cell suspension, 100 ul / well.
3. For PM 9+
   a. Add 13.92 ml of cell suspension to 130.08 ml of PM9+ inoculating fluid.
   b. Inoculate PM 9-20 with this cell suspension, 100 ul / well.

SECTION III. Incubation and Data Collection

1. Enter worksheet data into the OmniLog Software.
2. Load the PMs into the OmniLog.
3. Incubate all PMs in the OmniLog at **30°C**. for **36 to 72** hours
4. Remove plates from OmniLog.
5. Download the data for analysis at another computer.