PM Procedures for *S. cerevisiae* and other Yeast

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 1-10, 21-25	Biolog	12111, 12112, 12121, 12131, 12141, 12181, 12182, 12183, 12161, 12162, 12221-12225
IFY-0 Base Inoculating Fluid (1.2x)	Biolog	72231
SC Amino Acid Mixture	Sunrise Science Products	1300-030
YNB w/o Amino Acids	Fisher Scientific	DF0919-15-3
Biolog Universal Yeast (BUY) agar plates	Biolog	71005
Biolog Redox Dye Mix D (75x)	Biolog	74224
Biolog Redox Dye Mix F (75x)	Biolog	74226
Biolog Redox Dye Mix H (75x)	Biolog	74228
Biolog Redox Dye Mix E (100x)	Biolog	74225
menadione sodium bisulfite (optional)	Sigma	M5750
D-glucose	Sigma	G8270
turbidity standard, 85% T	Biolog	3431
sterile cotton swabs	Biolog	3021
sterile pipet tips	Biolog	3001
sterile reservoirs	Biolog	3102
sterile 20 x 150 test tubes	E+K Scientific	266B
sterile 120 ml plastic vial	Capitol Vial Corp.	1-24-786
sterile sealing tape for microplates (optional)	Sigma	Z369667
Other chemicals that may be needed		
D-pantothenate hemicalcium	Sigma	P2250
thiamine HCl	Sigma	T4625
L-histidine HCl monohydrate	Sigma	H8125
L-leucine	Sigma	L8000
L-lysine HCl	Sigma	L8662
L-methionine	Sigma	M5308
L-tryptophan	Sigma	T8941
adenine HCl	Sigma	A9795
uracil	Sigma	U0750

Section B. Preparation of Stock Solutions and Inoculation Fluids

Table 3.a. Composition and Preparation of 48x Yeast Nutrient Supplement (NS)[a]

Add ingredients to water and Q.S. to 100 ml. Filter sterilize and store at 4° C.

Ingredient	1x Conc.	480x Conc.	Formula Weight	Grams/ 100 ml	Nutrient Supplement
D-pantothenate	1.2uM	576uM	238.3	.0137	10ml
thiamine HCl	0.25uM	120uM	337.3	.0040	10ml
L-histidine HCl	10uM	4.8mM	209.6	0.101	10ml
L-leucine	100uM	48mM	131.2	0.630	10ml
L-lysine HCl	50uM	24mM	182.7	0.438	10ml
L-methionine[b]	25uM	12mM	149.2	0.960	10ml
L-tryptophan	25uM	12mM	204.2	0.245	10ml
adenine HCl	50uM	24mM	171.6	0.412	10ml
uracil	30uM	14.4mM	112.1	0.161	10ml
sterile water					10ml
Total					100ml

[a]The nutrient supplement provides all essential nutrients that are not provided elsewhere. It should include supplements for all auxotrophies of the strain being tested. If one or more of the listed supplements are not necessary for a strain's auxotrophic requirements, it should be omitted and replaced with sterile water.

[b]0.12mM pyridoxine may be used in place of L-methionine for met15Δ0 strains. Methionine contains sulfur and can interfere with sulfur source testing in PM4, whereas pyridoxine does not.

Table 3.b. Additional Solutions to Prepare

Add ingredients to water and Q.S. to 100 ml. Filter sterilize and store at 4° C.

Ingredient	1x Conc.	Stock Conc.	Formula Weight	Grams/ 100 ml
SC Medium	-	(1.2x)	-	0.804 YNB + 0.24 SC mixture
D-glucose	100mM	2.4M (24x)	180.2	43.248
menadione sodium bisulfite	1uM	1mM (1000x)	276.2	0.0276

Table 4. Recipe for 1x PM Inoculating Fluids from Stock Solutions [a]

PM Stock Solution	PM 1,2 (ml)	PM 3-8 (ml)	PM9+ (ml)
IFY-0 (1.2x)	20.0	60.0	-
SC Medium (1.2x)	-	-	70.0
Dye mix D, F, or H (75x)	0.32	0.96	-
Dye mix E (100x)	-	-	0.84
D-glucose (24x)	-	3.0	3.5
sterile water	3.18	6.54	7.91
cell suspension (48x)	0.5	1.5	1.75
Total	24.0	72.0	84.0

[a] For PM1-8, we recommend Dye mix D for *Saccharomyces, Schizosaccharomyces, Zygosaccaharomyces, Kluyveromyces,* and *Dekkera,* Dye Mix F for *Candida albicans*, Dye Mix H for *Pichia,* and Dye Mix E with 1uM menadione sodium bisulfite for *Cryptococcus* and *Rhodotorula*. Menadione sodium bisulfite is prepared at 1000x as shown in Table 3.b. and 12ul is added to every 12ml. Dye mix E usually works best for PM9+.

SECTION II: PROCEDURES for PM INOCULATION

Section A. Cell Suspension Preparation and PM Inoculation

Preparation of PM Inoculating Fluids

1. Prepare a sterile test tube containing 20 ml of sterile water (for prototrophs) or NS solution (for auxotrophs).
2. Prepare inoculating fluids as specified in Table 4.
3. Dispense inoculating fluids into vials as diagrammed in Figure 1 (see below).

Inoculation of PM Panels

Step 1: Prepare Cell Suspension

Grow the yeast on a BUY agar plate or an alternative agar medium by streaking for isolated colonies and allow it to grow for at least 24 hours at 26-37 °C. Subculture a second time. Remove cells from the BUY plate using a sterile swab and transfer into a sterile capped tube containing 20 ml of sterile water for prototrophic strains, or NS solution for auxotrophic strains. Stir the cell suspension with the swab to obtain a uniform suspension. To avoid bubble formation, do not vortex or mix turbulently. Check the turbidity of the suspension using a Biolog turbidimeter; add cells to achieve 62% T (transmittance).

Step 2: Inoculate PM 1, 2

Add 0.50 ml of cell suspension to 23.5 ml of PM1,2 inoculating fluid. Inoculate PM 1 and PM 2 with this cell suspension, 100 µl / well.

Step 3: For PM 3 - 8

Add 1.50 ml of cell suspension to 70.50 ml of PM3-8 inoculating fluid. Inoculate PM 3-8 with this cell suspension, 100 µl / well.

Step 4: For PM 9+

Add 1.75 ml of cell suspension to 82.25 ml of PM9+ inoculating fluid. Inoculate PM 9-10, 21-25 with this cell suspension, 100 µl / well.

Step 5: Sealing the Plates

For strains needing prolonged incubation it may help to seal the plates with plastic tape (sterile 1 mil polyester) to keep the wells from drying out. Taping sometimes is also effective at reducing the background color in the negative wells and it prevents cross-well spreading of volatile chemicals (e.g., ethanol, acetic acid, ammonia), mycelia, and spores.

SECTION III: INCUBATION AND DATA COLLECTION

1. Enter worksheet data into OmniLog Software.
2. Load the OmniLog.
3. Incubate all PMs in OmniLog at 26-37°C for 24-72 hours (e.g. 30°C, 48hr).
4. Remove plates from OmniLog and store at 4°C.
5. Collect the data for analysis.

Figure 1. PM Procedures for *S. cerevisiae* and other yeast