Yeast Plates

Hahn Lab
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Amino Acid Mix (40X) Use 0.6 g/liter
Tyrosine 2 g
Arginine 4 g
Serine 2 g
Valine 2 g
Threonine 4 g
Isoleucine 2 g
Phenylalanine 2 g
Aspartic Acid 2 g
Proline 2 g

Glucose Complete Plates (1 liter):
1.7 g Yeast Nitrogen base -amino acids and ammonium sulfate
5.0 g Ammonium Sulfate
1 liter H2O
pH to 7.0 using NaOH

Add:
0.6 g Amino Acid mix (mix well before adding)
20g Bacto-agar (Difco)

Autoclave 25 min.

Add:
10ml (10/mg/ml) of any other required amino acids
10ml 0.4% Adenine sulfate in 0.1M HCl if required
10 ml 0.2% Uracil if required
50 ml 40% glucose

YPD Plates (1 liter):
Yeast Extract 10 g
Bacto peptone 20g
Bacto-Agar 20g
H2O 1 liter

Autoclave 25 min

Add:
50 ml 40% glucose
5 ml 0.4% Adenine sulfate in 0.1 M HCl if required

Add 10 ml 0.2% Uracil (only if plates will be used for tetrad dissection)

5-FOA plates (0.5 liter):

Agar:
15 g Bacto-agar
300 ml H20 in 500 ml flask
Autoclave 25 min.

FOA mix:
0.85 g Yeast Nitrogen base - amm. sulfate and amino acids
2.5 g Ammonium sulfate
0.3 g amino acid mix (from above)
25 ml 40% glucose
17.5 mg Uracil
0.5 g 5-FOA
Any other required amino acids
225 ml H2O

Microwave the above mixture for about 3 min until about 65 degrees. Stir on stir plate until dissolved (~10-15 min). Filter through 0.2 micron bottle top filter into sterile bottle. Add to autoclaved agar. Mix well and pour plates. Do not pH the plates as this will inactivate the 5-FOA.

Antibiotic yeast plates:

G418 Plates

Current batches of Geneticin powder are not sterile. To sterilize, dissolve at 100 mg/ml H2O in a small beaker (eg., 0.5g G418 in 5 ml H2O). Filter sterilize using a syringe tip filter and add to warm agar.

Use G418 for yeast containing the KanMX allele. Add to plates at 500 micrograms/ml for selection of gene disruptions using yeast transformation. Can use at 200 micrograms/ml for checking cells already containing resistance to G418.

Hygromycin B Plates
Use Hygromycin B at 200 microgram/ml. Add 4 ml 50 mg/ml (Roche) per liter

Nourseothricin (NAT) Plates

Use clonNAT (Werner BioAgents, Germany) at 100 micrograms/ml using a 1000x stock kept at -80 degrees.