Yeast Extract-Peptone-Glycerol +Antifolates (YPGly+ AF) Media

William G. Alexander, Drew T. Doering, and Chris Todd Hittinger

Abstract

This recipe is from:
Alexander WG , Doering DT , and Hittinger CT (2014) High-Efficiency Genome Editing and Allele Replacement in Prototrophic and Wild Strains of Saccharomyces. Genetics 198:859-866; doi:10.1534/genetics.114.170118

See the full protocol for step-by-step YPGly+ AF preparation instructions.

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Guidelines

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1) Add the following components to a 2-L Erlenmeyer flask:
-10 g yeast extract
-20 g peptone
-5 g sulfanilamide
-50 mg hypoxanthine
-18 g agar
-900 mL ddH2O

Mix to dissolve as much as possible (agar and sulfanilamide won't dissolve until heated).

2) Autoclave for no more than 20 minutes on a liquid cycle.

3) Once autoclaved, cool to 50° in a water bath, then add the following and mix:
-5 g thymidine
-200 mg methotrexate
-100 mL 50% (v/v) glycerol, sterilized
(**NOTA BENE**: the standard operating procedure for adding compounds after autoclaving is to dissolve them in a
solvent, filter, then add to the media; this generally is difficult or impossible for methotrexate and

thymidine due to
the amount required. For the last two years, I've been adding the solid chemicals directly to the cooled media, and
I've never had contamination. I suspect that the extreme conditions prevent microbial growth. Also, both
methotrexate and thymidine are sensitive to heat, so take care to not add them early.)

4) Pour ~20 mL into plastic petri dishes and allow to set. You have now made YPGly +AF media

Protocol

Step 1.
10 g yeast extract

Step 2.
20 g peptone

Step 3.
5 g sulfanilamide

Step 4.
50 mg hypoxanthine

Step 5.
18 g agar

Step 6.
900 mL ddH2O