

Protocol for 5-Fluoroorotic Acid (5-FOA) Media

Introduction

5-Fluoroorotic Acid (5-FOA) is widely used in the field of yeast molecular genetics. The toxicity of 5-FOA to yeast cells with a functioning URA3 gene which encodes orotidine-5'-P decarboxylase (OMP decarboxylase), makes it especially useful for the selection and identification of mutant yeast strains. 5-FOA is most often applied with *Saccharomyces cerevisiae* (URA3), although it has been used in other strains including *Schizosaccharomyces pombe* (URA4 and URA5), *Candida albicans* (URA3) and the bacterium *E. coli* (pyrF). The use of 5-FOA in the yeast 2-hybrid system allows for the construction of activation domain hybrid libraries to identify protein-protein interactions. Here, we describe a general protocol for the preparation of 5-FOA media.

Materials

- [5-Fluoroorotic acid monohydrate \(GoldBio Catalog # F-230\)](#)
- [DMSO, Sterile Filtered \(GoldBio Catalog # D-361\)](#)

Yeast media

- Sucrose
- NaNO₃
- K₂HPO₄
- MgSO₄·7H₂O
- KCl
- FeSO₄·7H₂O
- Agar
- Molecular biology grade water

Storage and Handling

- Store 5-Fluoroorotic acid monohydrate at -20°C.
- Protect from light.
- This product may be shipped on blue ice and should be stored at -20°C immediately upon arrival. When stored under the recommended conditions and handled correctly, this product should be stable for at least 1 year from the date of receipt.

Method

Preparation from powder.

1. Make 400 mL of standard yeast synthetic media (see below for details) and add 1 g of [5-Fluoroorotic acid \(GoldBio Catalog # F-230\)](#) in powder form and sterilize by filtration. 5-Fluoroorotic acid powder can also be added after autoclaving once media has cooled to 55°C.
2. Autoclave agar/water (concentration 20g/600 mL) and allow to cool to 55°C.
3. Add the cooled 5-Fluoroorotic acid media to the agar/water, mix and pour plates.

In 100x 5-FOA solution

1. Dissolve 100 mg of 5-Fluoroorotic acid ([GoldBio Catalog # F-230](#)) in 1 ml of DMSO for a final concentration of 100 mg/ml.
2. Make a standard yeast synthetic agar containing media using a preferred method.
3. Autoclave to sterilize.
4. Let cool to 55°C and add aminoacids, sugars or other heat sensitive components.
5. Add 10 ml of 100x 5-Fluoroorotic acid solution per liter, mix and pour plates.

Czapek's Solution Agar

This is a synthetic medium commonly used in mycological laboratories.

Component	Amount
Sucrose	30.0 g
NaNO ₃	3.0 g
K ₂ HPO ₄	1.0 g
MgSO ₄ · 7H ₂ O	0.5 g
KCl	0.5 g
FeSO ₄ · 7H ₂ O	0.01 g
Agar	15 g
Molecular biology grade water	Up to 1000 ml

Associated Products

- [DMSO, Sterile Filtered \(GoldBio Catalog # D-361\)](#)