Protocol

TD-P Revision 2.0

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5-Fluoroorotic Acid (5-FOA) medium

Introduction

URA3 encodes orotidine 5-phosphate decarboxylase which can convert 5-FOA to 5-fluorouracil, a compound that causes cell death. Thus, when a yeast strain is added to this medium, it is possible to determine if the strain contains the URA3 gene based upon cell viability.

Materials

- 1 g 5-FOA (GoldBio Catalog # F-230) (0.1% w/v final)
- 500 mL molecular biology grade water
- 6.7 g Yeast Nitrogen Base (YNB) - amino acids (0.67% w/v final)
- 50 mg Uracil (50 µg/ml final)
- 20 g Dextrose (2% w/v final)
- Desired media supplements

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

1. Combine 5-FOA, molecular biology grade water, and a stir bar in a 1 L flask.

2. Autoclave or stir over low to medium heat for approximately 1 hour to dissolve 5-FOA.

3. Add sterile components (YNB, Uracil, Dextrose and supplements) once media has cooled down to approximately 55°C.

References

Green, Simon R.; Charles M. Moehle, *Current Protocols in Cell Biology*. John Wiley & Sons, Inc. 2003.

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