Nov 17, 2025

SD 5-Foa Plates For Yeast Counter Selection

DOI
https://dx.doi.org/10.17504/protocols.io.yxmvmbw7bg3p/v1

Ryan Tan[1]
[1]NTU


Ryan Tan

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Protocol Citation: Ryan Tan 2025. SD 5-Foa Plates For Yeast Counter Selection. **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvmbw7bg3p/v1

Protocol status: Working
We use this protocol and it's working

Created: September 18, 2025

Last Modified: November 17, 2025

Protocol Integer ID: 227560

Keywords: foa plates for yeast counter selection, yeast counter selection, foa plate, yeast, counter selection of ura3, prepartation of sd, sd, counter selection

Abstract

This protocol is for the prepartation of SD 5-Foa plates to use for the counter selection of URA3 in yeast.

Materials

6.7g/L **Yeast Nitrogen Base (No amino acids, no amonium sulfate)**
5g/L **Ammonium Sulfate**
2% (w/v) **Glucose**
2% (w/v) **Agar**
1g/L **5-Fluoroorotic Acid**
50mg/L **Uracil**
(According to Manfucaturers specifications) **-Ura Drop out Mix**

Before start

Integrated marker cassette of ‘loxp-kanMX-URA-loxp’ is proven to be removed by the low-occurrence event of mitotic recombination between the repeated loxp-loxp, and positive colonies could be selected out by marker loss upon URA counter-selection in a one-step process.

Preparation of SD 5-Foa plates

1 In a clean bottle, add **Yeast Nitrogen Base (No amino acids, no amonium sulfate), Ammonium Sulfate and glucose.**

2 In another clean bottle, add **Agar.**

3 Add DI H2O to both bottles. (If preparing for 100mL, suggest to add 60mL to bottle containg **Agar**, and the rest of the 40mL to the other bottle)

4 Autoclave at 121^{o}C for 15minutes.

5 Once cooled to around 50^{o}C, add **5-Fluoroorotic Acid, Uracil, and -Ura Drop out Mix** to the bottle containing **Yeast Nitrogen Base (No amino acids, no amonium sulfate), Ammonium Sulfate and glucose.**

6 Filter Sterile using 0.22um filter of contents in above bottle, into bottle containing **Agar.**

7 Dispense agar medium into petri dish (Recommend around ~15mL per petri dish)

1 day before experiment: 5mL culture of Cells

8 Innoculate transformed yeast cells from YPD agar plates (or whatever medium used) into 5mL of YPD. Remember to add markers in 5mL YPD.

9 Let it grow overnight in 30^{o}C incubator, with shaking of 200rpm.

Day of Experiment: Plating onto SD 5 Foa Agar Plates

10 Centrifuge overnight culture at 1,100 xG for 5minutes.

11 Remove supernatant and wash cells with 5mL of sterile DI water.

12 Repeat washing step once more.

13 After final washing step, resuspend cells in 1mL of DI water

14 Plate 150 - 200uL of cells onto SD 5-Foa agar plates. (try not to plate too much cells onto agar plate, if not it will be overcrowded and cells will not grow)

15 If counter selection is successful, colonies should appear after 2-3 days.

2-3 Days After the Experiment: Confirm Counter Selection/ Marker Excision

16 Inoculate colonies from SD 5Foa plate into 5mL YPD medium.

17 Let it grow overnight in 30^{o}C incubator, with shaking of 200rpm.

18 Plate 100 - 150uL of cells onto YPD agar with markers.

19 If counter selection and marker excision is successful, there should not be any colonies seen on the plates.

Protocol references

DOI: 10.1038/ncomms12851