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## Pre-Imaging Solid Growth Medium - Yeast

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**We use this protocol and it's working**

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## Abstract

This protocol describes the steps to prepare solid culture medium for *Saccharomyces cerevisiae*.

This solid medium is used for cultures that are going to be investigated by fluorescence imaging. Of that reason the medium is prepared with an abundance of Adenine to reduce auto-fluorescence [1].



## Materials

### SC-Ura Powder

Sunrise Science Products

Cat#: 1306-030

Lot#: 23K3083

Exp: 10/2027

### Yeast Nitrogen Base Without Amino Acids

Sigma Life Science

Cat#: Y0626-250G

Lot#: SLBG0555V

### Glucose

Sunrise Science Products

Cat#: 1907-1kg

Lot#: 3A0036

### Agar

Sunrise Science Products

Cat#: 1910-500

Lot#: 3B0104

### Sodium Hydroxide

Fisher Scientific

Cat#: S318-500

Lot#: 130802

### L-Adenine

Sigma Life Science

Cat#: A-9795

Lot#: 33H12895

### Deionized Water

### Equipment:

500 ml laboratory bottle with screw cap

1ml pipette

50 ml pipette

stirring hot plate

magnetic stirring bar

micro scales



autoclave  
thermometer  
10cm polystyrene Petri dishes (20 pieces)  
4°C fridge

## Before start

Have the following solutions premixed:

**Glucose 20%** 500 ml solution:

Concentration: 200 g/l

mix 100 g Glucose in 500 ml deionized water (ddH<sub>2</sub>O)

**Sodium Hydroxide 1M** solution 50 ml:

Concentration: 39.997 g/l

mix 1.99985 g NaOH into 50 ml ddH<sub>2</sub>O

**Adenine 100x** 100 ml solution:

Concentration 3 g/l

mix 0.3 g Adenine in 100 ml ddH<sub>2</sub>O

**Optional:**

**SC-xx 10x** 100ml solution:

Concentration: 19.2 g/l

mix 1.92 g into 100 ml ddH<sub>2</sub>O

**YNB 20x** 100ml solution:

Concentration: 134.4 g/l

mix 13.44 g into 100 ml ddH<sub>2</sub>O



## 1 Compound medium for autoclave

2m

### STEP CASE

#### Medium preparation with pre-resolved components

13 steps

This version of the protocol shows the preparation of the medium from SC-XX 10x and YNB 20x solutions.

- 1.1 Fill a 500 ml flask with  369 mL H<sub>2</sub>O.

Add a magnetic stirring bar and place the flask on a stirring hot plate.

- 1.2 Add  25 mL YNB 20x solution (Yeast Nitrogen Base with Ammonium Sulfate without Amino Acids).

- 1.3 Add  50 mL SC-XX 10x solution.

#### Note

In regard to cover all optional dropout media the amino acid base holds the notification - xx, where xx stand for the amino acid(s) that is as selection factor, missing in the medium.

- 1.4 Add  10 g Agar

- 1.5 Add  5 mL Adenine 100x solution

#### Note

The additional Adenine is supposed to repress the Adenine synthesise to reduce a possible accumulation of red pigment [1].

- 1.6 Add  1 mL NaOH 1 molar solution.

#### Note

This is essential for the solidification of the medium!



2 Autoclave for 00:15:00 at 121 °C .

#### Note

Remove the stirring bar before going to autoclave.

3 Cooling and plating

3.1 Add a sterile stirring bar and thermometer. Let the medium stir to homogenize its temperature, while cooling.

3.2 When the medium cooled down to around 80 °C add 50 mL sterile Glucose 20%.

3.3 When the medium cooled down to 60 °C , plate 25 mL per 10 cm Petri dish (~20 dishes).

3.4 Let the medium solidify and cool down to Room temperature .

4 Seal the prepared dishes in a plastic bag to prevent condensation and store them in 4 °C fridge.

#### Note

The agar plates can be store in the 4°C fridge for 2 to 3 months.

## Protocol references

[1] Kokina, Agnese et al. "Adenine auxotrophy—be aware: some effects of adenine auxotrophy in *Saccharomyces cerevisiae* strain W303-1A." *FEMS yeast research* 14.5 (2014): 697-707.  
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