Yeast Extract Peptone Dextrose (YPD) Medium

Instructions

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Applications

Yeast Peptone Dextrose (YPD) medium is formulated by combining Yeast Peptone medium (YP) with Dextrose (D-glucose). YPD medium is commonly used for the cultivation of fungal species such as *Saccharomyces cerevisiae* and *Pichia pastoris*, and has also been employed in studies involving the exploration of *Streptomyces*.

Components	per litre
Yeast extract, premium grade	10 g
Bacteriological Peptone (Bovine-origin enzymatic digest)	20 g
Glucose	20 g

Preparation

Dissolve 50.0 g of media powder in 1000 mL of distilled water. Heat to dissolve the medium completely. Sterilise by autoclaving at 115°C for 30 minutes. Aliquot as needed.

Caution

Monosaccharides are prone to undergoing the Maillard reaction when exposed to amino acids at high temperatures. Therefore, if the user wants to maximise the bacteria growth, sterilising at 121°C for 15 minutes is not advisable. Instead, if circumstances allow, a 0.22 µm filtration can be used as an alternative to autoclave.

YPD Agar Preparation Protocol

Materials Required:

- Clean 1 L GL45-cap reagent bottles, x 2
- Milli-Q water or double-distilled water, 1 L
- Autoclave equipment
- Agar powder
- YPD (Yeast Extract Peptone Dextrose) media powder
- Sterilised plates (glass or plastic)
- Analytical balance (scale)
- 1 L beaker
- 1 L graduated cylinder
- 5 M HCl and 5 M KOH stock solutions for pH adjustment
- Clean bench (either vertical or laminar flow)
- 0.22 µm filter and vacuum pump (optional)

Media preparation:

Weigh 50 g of YPD media powder and 3~4 g of agar powder. Add to a beaker and dissolve thoroughly in MQ water. Adjust the final volume to 1 Litre using a graduated cylinder.

pH Adjustment (Optional)

Measure the pH of the solution and adjust to approximately 6.5 using 5 M HCl or 5 M KOH if necessary. This step may be skipped if pH sensitivity is not critical.

Peptide precipitation might occur after autoclave if the pH of the stock solution is high (e.g., pH 9–10). This precipitation happens at the peptides' specific isoelectric point (pI) and is not harmful or significantly impactful to bacterial culturing, especially for yeast, like Saccharomyces cerevisiae and Pichia pastoris, which can secrete a group of proteases capable of digesting the precipitated peptides and redissolving them.

Sterilisation

Dispense the media into two 1 L GL45-cap reagent bottles (~500 mL per bottle). Do not overfill the bottle.

Secure the bottle caps, leaving them one turn loose from fully tightened. Optionally, cover bottles with autoclave bags.

Autoclave at either:

- 115 °C for 30 minutes (preferred if available)
- 121 °C for 15 minutes (note: may slightly darken the media but typically does not impact yeast growth)

Plate Pouring

Once autoclave is complete, allow the media to cool to 60~65 °C.

On a clean bench, pour the hot liquid agar into sterilised plates* to a recommended thickness of 5~8 mm. The entire pouring process should be carried out swiftly and steadily in one continuous session to minimise contamination and ensure uniform solidification (generally, 500ml agar media can make about 25~30 pieces of 8cm plates).

**Wear thick rubber gloves or double-layer gloves to handle hot bottles safely.*

Partially cover plate lids in a tilted manner to prevent water condensation on the lids during solidification.

Storage

Once agar has fully solidified (approx. 15~20 minutes), fully close the plate lids and invert the plates to prevent condensation water from dripping onto the agar surface.

Stack plates carefully, seal in plastic bags, and store at 4 °C for long-term use.

Note: Even at 4 °C, cold airflow in refrigerators can freeze and damage plates—use a Styrofoam box when storing is recommended.

Pre-use Handling

Before plating the strain, allow stored plates to equilibrate to room temperature for about 30 minutes.