# Microsome preparation

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## Abstract

Protocol to extract the inner membranes from yeast cells

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## Protocol

### Yeast cell growth (Steps 1-)

**Step 1.**
Streak out the strain from a -80 °C glycerol stock on a YPD-plate and incubate at 30 °C for 2-3 days.

**Step 2.**
Isolate a single colony and streak it out on a YPD-agar and streak it out on a YPD-agar plate and incubate 2-3 days at 30 °C.

**Step 3.**
Inoculate a 10 ml YPD medium in a 100 ml flask with a loop of cells from a fresh plate and grow culture approximately for 18-20 h at 28 °C in a shaker (180 rpm).

**Step 4.**
Inoculate 2-4 x 100 ml YPD (optional: with 2.5 % DMSO, this makes the cells to form more inner membranes) at OD 0.1 in 1 l baffled flask and grow at 28 °C, 150-160 rpm for approximately 12-14 h until reaches to OD 2-2.5.

**Step 5.**
Harvest cells by centrifugation at 8,000 rpm for 10 min at 4 °C.

**Step 6.**
Discard supernatant and resuspend pellet with ice-cold 1x PBS, wash the pellets one more time and measure the biomass.

### Microsome preparation (steps 7-1)

**Step 7.**
ALL THE FOLLOWING STEPS FOR PREPARATION OF MICROSOMES SHOULD BE CARRIED OUT AT 4 °C!

**Step 8.**
Resuspend the pellet from previous step with ice-cold lysis buffer with the ratio 1:3 (w:v) and transfer it into a new tube. From this step forward all the buffer should contain 1 mM DTT, 10 mM PMSF, PIC.

**Step 9.**
Add equal amount of glass beads to the cell suspension and vortex at the highest speed for a min and cool it down on ice for 1 min, repeat it 10-12 times. Check the cells under microscope for the disruption efficiency. This step is recommended to be carried out in a cold room.

**Step 10.**
Add fresh 10 mM PMSF after breaking of the cells is completed. (PMSF is unstable in aqueous

solutions.)

**Step 11.**
Transfer broken cell suspension into a new tube, avoid transfering glass beads.

**Step 12.**
Wash glass beads with the lysis bugger until most of the broken cells were removed from glass beads, collect all of samples and proceed to centrifugation step.

**Step 13.**
Centrifuge at 17,000 g for 10 min to remove the cell debris and unbroken cells.

**Step 14.**
Pour the supernatant into unltracentrifuge tubes and centrifuge for 1 h at 160,000 g at 4 °C.

**Step 15.**
Discard the supernatant and mix microsome pellets with 1x PBS containing 5 % glycerol with the ratio of 1:10 (w:v). First add 1-2 ml of the buffer and resuspend it with P-1000 pipette until the pellet falls apart to small fragments, using a trimmed pipette tip will help to avoid clogging the tip.

**Step 16.**
Transfer the microsome suspension into a pre-chilled douncer and add the rest of the buffer. Gently homogenize the mix with 10-12 strokes.

**Step 17.**
Measure absorbance at 280 nm.