

Standard inoculation conditions - Rosanne Hertzberger, October 2017, update January 2020

We use 20% glycerol stocks. We do not have dry ice so we use regular ice boxes to transfer the tubes from the -80 to the bench or into the anaerobic chamber and back, which means they become quite a bit warmer/softer compared to using dry ice. We have several different tubes of the same strain and regularly make new stocks.

Aerobic inoculation:

Preparing the plates occurs on the bench, aerobically. With disposable inoculation loop, we transfer a small scoop of material from the glycerol stock and spread it out over an NYCIII agar plate. These plates are placed in an Oxoid transparent jar. After closing the jar we apply three cycles of: pulling a vacuum and filling with $N_2+5\%CO_2$ gas. We incubate at 37° degrees.

The plates must be used between 48 hours and 7 days. Plates that are left anaerobically at room temperature were found to not lead to good precultures for *Lactobacillus crispatus*, the plates are therefore kept at 37° degrees until use. To inoculate a liquid preculture we add a “scoop” from these plates to 2 mL of NYCIII glucose medium in 12 mL Greiner tubes (ref 164162). We close the tubes with the caps but do not screw them tight to leave air flow to allow for the liquid to equilibrate with the anaerobic atmosphere. Preparing precultures from a single colony has not given good results previously.

Again, this preculture is incubated using the Oxoid transparent jar made anaerobic by applying vacuum and filling with $N_2+5\%CO_2$ gas in three consecutive cycles.

We regularly let the preculture incubate for 4 days (96 hours), inoculate all strains on Thursday and use them on the next Monday. This is twice as long as the recommended growth times of the slowest growing bacteria in my practice (*L. iners* DSM strain). Optimally, we would use 48 hours however, this is impractical due to the experiments we are running and the time we have. Furthermore, we have not experienced any problems using this preculturing time.

(Previously, when we had growth problems with the plates, we have also inoculated directly into liquid culture, 2 mL of standard NYCIII glucose medium and regularly checking for homogeneous colony morphology on plates.)

Anaerobic inoculation:

The same time periods, media, temperature and practices as above, yet here we use an anaerobic chamber filled with $N_2+5\%CO_2$ gas. Plates and media are equilibrated for at least 24 hours prior to use in the chamber. The plates and precultures are transported outside of the chamber in smaller steel containers and are incubated in a 37° degrees stove.

Standard glycogen growth assay

We have used NYCIII medium supplemented with either 0.5% glycogen or nothing (water, negative control) or 0.5% glucose as a positive control. To this end we use deepwell 96 well plates (individually wrapped, sterilized) or sterile eppendorf tubes and have pipetted 100 uL of a 5% glucose solution (in water) or 5% glycogen solution (in water) or water and 900 uL of a 1.1x NYCIII medium where we have left out the glucose (see separate protocol for edium).

In case of the deepwell plates, they are covered with lids that are not airtight. We incubate the plates anaerobically, without shaking, at 37°C, in the Oxoid jar as described above. After

48 hours we mix by pipetting up and down with a 1 mL pipet and dilute the culture 10x with PBS in a flatbottom 96-well plate to measure the cell density in the plate reader at OD600.

MRS agar plate

Dissolve 51 gram of Sigma-Aldrich MRS broth (nr. 69966) in 1L of water, add 1 gram/L Tween80. stir until dissolved. Add 10 g/L agar and autoclave at 121°C for 20 minutes. Poor ~20 mL sterilely into petri dishes in the flow cabinet. Let them solidify and dry. Store at 4°C.