Special Instructions

Cultivation instructions for *Helicobacter*

If you have ordered an **Active Culture** of *Helicobacter*, you will receive a vial containing a blood-agar slant which is covered with liquid. Most likely, you will not be able to see single colonies or growth. Most cells are situated in the liquid phase and are well visible under the microscope (1000 x magnification; phase contrast). Depending on the duration of transport (in most cases cultures are shipped on Wednesday) you will see many coccoid cells which represent the dying form of the bacterium.

It is of utmost importance to subculture immediately after arrival! Conditions in the vial are not optimal for the cells and they should be removed as quickly as possible. Remove the liquid completely, rinse the vial with 0.5 ml medium (e.g. TSB, nutrient broth or BHI) and add **everything** onto **one moist** blood agar plate (see below).

If you have ordered an **ampoule** follow the instructions as given in Opening of Ampoules, solubilize the lyophilisate in 1 ml of medium (e.g. TSB, nutrient broth or BHI) and add **everything** onto **one** blood agar plate.
It is **imperative** to incubate the plate at 37°C in the gas phase that is recommended for the strain. Furthermore, it is recommended to add a moist paper towel to the container to ensure a sufficiently high air moisture.

Subculture *Helicobacter* on saturated plates only (if your plates are a little older or slightly dry, add about 1 ml of liquid medium – e.g. TSB, nutrient broth or BHI – onto each plate and let them soak up the liquid. When the plate does not take up any more liquid, it's okay). Some liquid should still be present on the plate the next day.

Incubate the plate lid-up in an anaerobic container equipped with a gas pack for *Campylobacter* or the respective gas phase at 37°C. Furthermore, it is recommended to add a moist paper towel to the container to ensure a sufficiently high air moisture.

The next day, in one drop of the liquid above the agar many living and active cells should be visible. If you do not need the culture to start your experiments immediately, you should prepare several plates from this first subculture to preserve a larger number auf cells. *Helicobacter* does not take passaging over several steps well.

Preservation: if you want to preserve cells it is of utmost importance to catch the timepoint of highest vitality. You should preserve cells only if they appear as curved rods and move around actively. Only these cells will survive storage satisfactorily. If many cells are present in the supernatant above the agar, you can dilute it with liquid medium before adding glycerol (20%; sterile) to achieve a final concentration of 10% glycerol. In general aliquots of 500 µl or 1 ml (depending on the cell density) are frozen, which are plated on **one** moist agar plate after thawing. At DSMZ cells are principally stored at -80°C, storage at -20°C is not recommended and should only be used for short periods of time (max. 2 months).