## Preparation of liquid media for strictly anaerobes at JCM (1/3)

JCM also provides strictly anaerobic archaea/bacteria such as methanogenic archaea and sulfatereducing bacteria. This recipe shows how we prepare liquid media for such strictly anaerobic archaea/bacteria.



An apparatus for the gassing anoxic gases (in the left hood) and a gas exchanger (gassing manifold) equipped with a vacuum pump (in the right hood)

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## Preparation of liquid media for strictly anaerobes at JCM (2/3)



Prepare a heat-stable solution (not containing the reducing agent, NaHCO<sub>3</sub>, vitamins etc.) in a flask. A few boiling stones may be thrown in it. Put the flask on a burner and bring to a boil. Until then, occasionally, shake the flask slowly.



Bubble anoxic gas through the solution after boiling and during cooling on ice. When the solution is cooled enough, add NaHCO<sub>3</sub> to the solution (alternatively, NaHCO<sub>3</sub> could be added after autoclaving).





Distribute the solution into culture vessels by a syringe under the anoxic gas (gassing through gassing needles) (A3). Stopper the culture vessels with butyl rubber stoppers while withdraw the gassing needles from culture vessels (A4).

## Preparation of liquid media for strictly anaerobes at JCM (3/3)



A gas exchanger (gassing manifold) equipped with anoxic gas cylinders and vacuum pump. It allows to replace gas phases in culture vessels sealed with butyl rubber stoppers by repeating the vacuum-gassing cycles.





Displace the dead air space of a syringe prior to use (C1-a). Then, remove a necessary amount of the reducing agent into the anaerobic syringe (C1-b).





Rapidly, add the reducing agent to the medium in an anerobic culture vessel (C1-c). When the medium is reduced (left, nonreduced; right, reduced, as judged by the resazurin color change to colorless), the medium is ready to be inoculated (C2).