

## Media for dechlorinators from the Löffler lab

<http://web.utk.edu/~microlab/LoefflerLab/Home.html>

### TSB (Tryptic Soy Broth Medium) + sugar source

1. Put 600 ml Distilled water into a 1.5 L round bottom bottle, and add the following:

Ingredient	1X (g/l)	1/2x (g/l)	1/4x (g/l)
TSB (no glucose)	27.5	13.75	6.875
Yeast Extract (1 g/L)	1	1	1
NaCl (total = 5 g/l)	--	2.5	3.75
Dextrose (or other)	2.5 –5	2.5 –5	2.5 –5

and bring the volume up to 1L (rinsing the powder residue off the inside flask neck).

2. Connect a condenser with cold running water flowing through it to the top of the round-bottom flask, and start a stream of N<sub>2</sub> flowing into the top. Set the flask onto a ceramic heating mesh on a ring stand over a bunsen burner to begin boiling the solution (drives off O<sub>2</sub>) under the nitrogen gas. This is the initial steps at producing an anoxic solution.

3. Boil for 10 minutes.

4. Cool the medium to room temperature (by suspending the flask in an ice bath) and then add 1 mM DTT as the reductant. (0.154 g/L TSB)

5. pH the solution to 7.2 (If done before adding DTT, the pH will drop)

6. Dispense to appropriate bottles/tubes, either with N<sub>2</sub> or N<sub>2</sub>+CO<sub>2</sub> atmosphere.