

CL Medium for *Chlorobaculum tepidum* TLS:

Chlorobaculum tepidum TLS (previously *Chlorobium tepidum* TLS), DSM 12025, ATCC 49652 was isolated from Travelodge Stream, Sulphur Bay, North Island, New Zealand in [[Wahlund et. al., 1991, *Arch. Microbiol.* 156: 81-90]].

Solutions:

A. Salts A (makes 1 L, can be refrigerated):

1. $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$: 3.5 g (mix in first or else it will not dissolve)
2. $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$: 0.64 g
3. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 10 g
4. NaCl : 20 g
5. Fill to 1 L mark with d.d. H_2O and then autoclave to sterilize. Refrigerate.

B. Salts B (makes 1 L, can be refrigerated):

1. $\text{NH}_4\text{CH}_3\text{COO}$ (anhydrous): 25 g
2. NH_4Cl (anhydrous): 20 g
3. $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$: 115 g
4. Fill to 1 L mark with d.d. H_2O and then autoclave to sterilize. Refrigerate.

C. CP Buffer (makes 1 L, can be refrigerated):

1. KH_2PO_4 : 25 g
2. MOPS Buffer: 105 g
3. Fill to 1 L mark with d.d. H_2O and then autoclave to sterilize. Refrigerate.

D. Resazurin solution (10 mg/mL in d.d. H_2O). Can be stored at room temperature.

E. Trace Elements Solution "CT" (makes 1 L, can be refrigerated):

1. $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$: 3 g
2. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$: 2 g
3. $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$: 0.19 g
4. $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$: 0.1 g
5. ZnCl_2 : 0.07 g
6. $\text{Na}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$: 0.036 g
7. $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$: 0.024 g
8. H_3BO_3 : 0.006 g
9. $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$: 0.002 g
10. Sterilize by passing through a 0.2 μm filter. Refrigerate.

F. Fresh NaHCO_3 solution (8 g/100 mL d.d. H_2O). Sterilize by passing through a 0.2 μm filter.

G. Fresh $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ solution (2.4 g/100 mL d.d. H_2O). Sterilize by passing through a 0.2 μm filter.

H. Vitamin B_{12} Solution (2 mg/mL in d.d. H_2O). Sterilize by passing through a 0.2 μm filter. Refrigerate.

To mix medium:

1. Mix into bottles, flasks, or carboys using the following scheme:

| | 0.5 L Media | 1 L Media | 2 L Media | 10 L Media | 12 L Media | 15 L Media |
|------------------|--------------------|------------------|------------------|-------------------|-------------------|-------------------|
| Salts A | 13 mL | 26 mL | 52 mL | 260 mL | 310 mL | 390 mL |
| Salts B | 13 mL | 26 mL | 52 mL | 260 mL | 310 mL | 390 mL |
| CP Buffer | 13 mL | 26 mL | 52 mL | 260 mL | 310 mL | 390 mL |
| Resazurin | 25 μ L | 50 μ L | 100 μ L | 500 μ L | 600 μ L | 750 μ L |
| Water | 440 mL | 880 mL | 1.76 L | 8.8 L | 10.6 L | 13.2 L |

2. Autoclave on 30- or 60-minute liquid cycle depending on vessel size. Allow to cool to room temperature.
3. Add the additional solutions using the following scheme:

| | 0.5 L Media | 1 L Media | 2 L Media | 10 L Media | 12 L Media | 15 L Media |
|--|--------------------|------------------|------------------|-------------------|-------------------|-------------------|
| Traces | 670 μ L | 1.33 mL | 2.66 mL | 13.3 mL | 16 mL | 20 mL |
| NaHCO₃ | 16.5 mL | 33 mL | 66 mL | 330 mL | 400 mL | 500 mL |
| Na₂S·9H₂O | 16.5 mL | 33 mL | 66 mL | 330 mL | 400 mL | 500 mL |
| Vit. B₁₂ | 13.5 μ L | 27 μ L | 52 μ L | 270 μ L | 325 μ L | 400 μ L |

4. Adjust pH to 7.0 (if necessary) with concentrated H₂SO₄ or Na₂CO₃.
5. Inoculate 1-2% with starter culture. Grow at 40 °C in high light for 2-4 days.