

Reference : Modified by ZEHNDER-STAU B study.

Note : Use fresh Milli-Q water to prepare stock solutions and culture medium.  
The stock solutions and culture medium are stored in the refrigerator.

### **List of the stocks solutions :**

- Z1 :  $\text{NaNO}_3$  at 85 g/L
- Z2 :  $\text{Ca}(\text{NO}_3)_2, 4 \text{H}_2\text{O}$  at 20 g/L
- Z3 :  $\text{K}_2\text{HPO}_4, 3\text{H}_2\text{O}$  at 40,62 g/L or ( $\text{K}_2\text{HPO}_4$  at 20 g/L)
- Z4 :  $\text{MgSO}_4, 7 \text{H}_2\text{O}$  at 25 g/L
- Z5 :  $\text{Na}_2\text{CO}_3$  at 21 g/L
- Z6 : Fe-EDTA solution (\*)
- Z7 : Trace elements solution (\*\*)

(\*) Fe-EDTA solution:

(a) EDTA- $\text{Na}_2, 2 \text{H}_2\text{O}$  solution :

In a 500 ml volumetric flask, weigh 323,52 mg of *EDTA- $\text{Na}_2, 2 \text{H}_2\text{O}$*  and adjust to volume with 500 ml Milli-Q water.

(b) HCl 0,1M :

In a 100 ml volumetric flask, transfer 1 ml of concentrated HCl and adjust to volume with Milli-Q water.

(c)  $\text{FeCl}_3, 6 \text{H}_2\text{O}$  solution :

In a 50 ml volumetric flask, weigh 1,351 g of  $\text{FeCl}_3, 6 \text{H}_2\text{O}$  then adjust to volume with 0,1 M HCl.

In a 1 L volumetric flask, transfer 500 ml of solution (a) and 10 ml of solution (c)  
Then adjust to volume with Milli-Q water.

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(\*\*) Trace elements solution :

In a 500 ml volumetric flask, weigh :

Element name	Mass weight (en mg)
H <sub>3</sub> BO <sub>3</sub>	1550
MnSO <sub>4</sub> , H <sub>2</sub> O / MnSO <sub>4</sub> , 4 H <sub>2</sub> O	858,5 / 1115
Na <sub>2</sub> WO <sub>4</sub> , 2 H <sub>2</sub> O	16,5
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> , 4 H <sub>2</sub> O	44
KBr	59,5
KI	41,5
ZnSO <sub>4</sub> , 7 H <sub>2</sub> O	143,5
Cd(NO <sub>3</sub> ) <sub>2</sub> , 4 H <sub>2</sub> O	77
Co(NO <sub>3</sub> ) <sub>2</sub> , 6 H <sub>2</sub> O	73
Cu SO <sub>4</sub> , 5 H <sub>2</sub> O	62,5
Ni(NO <sub>3</sub> ) <sub>2</sub> , 6 H <sub>2</sub> O / NiSO <sub>4</sub> (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , 6 H <sub>2</sub> O	72,9 / 99
Cr(NO <sub>3</sub> ) <sub>3</sub> , 9 H <sub>2</sub> O / Cr(NO <sub>3</sub> ) <sub>3</sub> , 7 H <sub>2</sub> O	20,3 / 18,5
NH <sub>4</sub> VO <sub>3</sub> / V <sub>2</sub> O <sub>4</sub> (SO <sub>4</sub> ) <sub>3</sub> , 16 H <sub>2</sub> O	5,5 / 17,5
Al(SO <sub>4</sub> ) <sub>2</sub> K, 12 H <sub>2</sub> O / Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> K <sub>2</sub> SO <sub>4</sub> , 24 H <sub>2</sub> O	237 / 237

Then adjust to volume with Milli-Q water.

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### Preparation of 1 L of Z culture medium :

N <sub>o</sub> of stock solution	Name of stock solution	Concentration of stock solution(g/L)	Volume of stock solution (mL)
Z1	NaNO <sub>3</sub>	85	5,50
Z2	Ca (NO <sub>3</sub> ) <sub>2</sub> , 4 H <sub>2</sub> O	20	2,95
Z3	K <sub>2</sub> HPO <sub>4</sub> , 3 H <sub>2</sub> O	40,62	1
Z4	MgSO <sub>4</sub> , 7 H <sub>2</sub> O	25	1
Z5	Na <sub>2</sub> CO <sub>3</sub>	21	1
Z6	Fe-EDTA	-	10
Z7	<u>Solution of trace-elements</u>	-	0,080

Complete to 1 liter with with Milli-Q water.

Then filter the Z medium through a filter of 0,22 µm in diameter in the laminar flow hood.

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