Référence : based on Z-formula, of CHU-10 (UTCC), vitamines F/2 (UTCC)

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 stocks solutions :

- ZSV1 : Na_2SiO_3, 5 H_2O at 21,2 g/L
- ZSV 2 : $NaNO_3$ at 85 g/L
- ZSV 3 : $MgSO_4$, 7 H_2O at 25 g/L
- ZSV 4 : K_2HPO_4, $3H_2O$ at 40,62 g/L or (K_2HPO_4 at 20 g/L)
- ZSV 5 : Ca $(NO_3)_2$, 4 H_2O at 20 g/L
- ZSV 6 : Na_2CO_3 at 21 g/L
- ZSV 7 : Fe-EDTA solution (*)
- ZSV 8 : Trace elements solution (**)
- ZSV 9 : Vitamin B12 solution *(Cyanocobalamine)* at 0,0001 g/L (***)
- ZSV 10 : Biotin solution at 0,01 g/L
- ZSV 11 : Thiamine solution at 0,1 g/L

(*) Fe-EDTA solution:

(a) *EDTA-Na_2, 2 H_2O solution* :
In a 500 ml volumetric flask, weigh 323,52 mg of *EDTA-Na_2, 2 H_2O* 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) *$FeCl_3$, 6 H_2O solution* :
In a 50 ml volumetric flask, weigh 1,351 g of $FeCl_3$, 6 H_2O 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.

	Editor	Checker	Approving
Name : Fonction : Visa :	Huguet Isabelle Technician	Chardon Cécile Research technician	Rimet Frédéric Ingineer

(**) Trace elements solution :

In a 500 ml volumetric flask , weigh :

Element name	Mass weight (en mg)
$MnSO_4$, H_2O / *$MnSO_4$, 4 H_2O*	858,5 / *1115*
Na_2WO_4, 2 H_2O	16,5
$(NH_4)_6Mo_7O_{24}$, 4 H_2O	44
KBr	59,5
KI	41,5
$ZnSO_4$, 7 H_2O	143,5
$Cd(NO_3)_2$, 4 H_2O	77
$Co(NO_3)_2$, 6 H_2O	73
Cu SO_4, 5 H_2O	62,5
$Ni(NO_3)_2$, 6 H_2O */ $NiSO_4(NH_4)_2SO_4$, 6 H_2O*	72,9 */ 99*
$Cr(NO_3)_3$, 9 H_2O */ $Cr(NO_3)_3$, 7 H_2O*	20,3 */ 18,5*
NH_4VO_3 */ $V_2O_4(SO_4)_3$, 16 H_2O*	5,5 */ 17,5*
$Al(SO_4)_2K$, 12 H_2O */$Al_2(SO_4)_3K_2SO_4$, 24 H_2O*	237 */ 237*

Then adjust to volume with Milli-Q water.

(***)Vitamin B_{12} solution *(Cyanocobalamine)* at 0,0001 g/L :
In a 100 ml volumetric flask, weigh 1 mg of vitamin B_{12}, then adjust to volume with Milli-Q water. This is the solution (a) .
Dilute 1ml of the solution (a) in a 100 ml volumetric flask with Milli-Q water.
This is the ZSV9 solution .

Preparation of 1 liter of (Z+Si+Vitamins) culture medium:

N_o of stock solution	Name of stock solution	Concentration of stock solution(g/L)	Volume of stock solution (mL)
ZSV1	Na_2SiO_3, 5 H_2O	21,2	2,65
ZSV2	$NaNO_3$	85	5,50
ZSV3	$MgSO_4$, 7 H_2O	25	1
ZSV4	K_2HPO_4, 3 H_2O	40,62	1,2
ZSV5	Ca $(NO_3)_2$, 4 H_2O	20	2,95
ZSV6	Na_2CO_3	21	1
ZSV7	Fe-EDTA solution	-	10
ZSV8	Trace elements solution	-	0,080
ZSV9	vitamin B12 solution	0,0001	0,1
ZSV10	Biotin solution	0,01	0,1
ZSV11	Thiamine solution	0,1	2

Complete to1 liter with with Milli-Q water.

Then filter the medium (Z+Si+Vitamins) through a filter of 0,22 µm in diameter in the laminar flow hood.