

Reference : for the macro-elements, this is the 10 of CHU modified by HUGHES, J.C & LUND, J.W.G.

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 :

- DV1 : Ca (NO₃)₂, 4 H₂O at 20 g/L
- DV2 : K₂HPO₄, 3H₂O at 40,62 g/L or (K₂HPO₄ at 20 g/L)
- DV3 : MgSO₄, 7 H₂O at 25 g/L
- DV4 : Na₂CO₃ at 21 g/L
- DV5 : Na₂SiO₃, 5 H₂O at 21,2 g/L or (Na₂SiO₃, 9H₂O at 58 g/L)
- DV6 : Fe-EDTA solution (*)
- DV7 : Trace elements solution (**)
- DV8 : Vitamin B₁₂ solution (*Cyanocobalamine*) at 0,0001 g/L (***)
- DV9 : Biotin solution at 0,01 g/L
- DV10 : Thiamine solution at 0,1 g/L

(*)Fe-EDTA solution :

(a) EDTA-Na₂, 2 H₂O solution :

In a 500 ml volumetric flask, weigh 323,52 mg of EDTA-Na₂, 2 H₂O and adjust to volume with milli-Q water.

(b) HCl 0,1M solution :

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

(c) FeCl₃, 6 H₂O solution :

In a 50 ml volumetric flask, weigh 1,351 g of FeCl₃, 6 H₂O then adjust to volume with 0,1 M HCl.

In a 1 L volumetric flask, transfer 500 ml of (a) solution and 10 ml of (c) solution 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 ₃ BO ₃	1550
MnSO ₄ , H ₂ O / MnSO ₄ , 4 H ₂ O	858,5 / 1115
Na ₂ WO ₄ , 2 H ₂ O	16,5
(NH ₄) ₆ Mo ₇ O ₂₄ , 4 H ₂ O	44
KBr	59,5
KI	41,5
ZnSO ₄ , 7 H ₂ O	143,5
Cd(NO ₃) ₂ , 4 H ₂ O	77
Co(NO ₃) ₂ , 6 H ₂ O	73
Cu SO ₄ , 5 H ₂ O	62,5
Ni(NO ₃) ₂ , 6 H ₂ O / NiSO ₄ (NH ₄) ₂ SO ₄ , 6 H ₂ O	72,9 / 99
Cr(NO ₃) ₃ , 9 H ₂ O / Cr(NO ₃) ₃ , 7 H ₂ O	20,3 / 18,5
NH ₄ VO ₃ / V ₂ O ₄ (SO ₄) ₃ , 16 H ₂ O	5,5 / 17,5
Al(SO ₄) ₂ K, 12 H ₂ O / Al ₂ (SO ₄) ₃ K ₂ SO ₄ , 24 H ₂ O	237 / 237

Then adjust to volume with milli-Q water.

(***) Vitamin B₁₂ solution (Cyanocobalamine) at 0,0001 g/L :

In a 100 ml volumetric flask, weigh 1 mg of vitamin B₁₂, then adjust to volume with milli-Q water. This is solution (a)

Dilute 1ml of solution (a) in a 100 ml volumetric flask, this is solution DV8.

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Preparation of 1 liter of DV culture medium:

N_o of stock solution	Name of stock solution	Concentration of stock solution(g/L)	Volume of stock solution (mL)
DV1	Ca (NO ₃) ₂ , 4 H ₂ O	20	2
DV2	K ₂ HPO ₄ , 3H ₂ O	40,62	0,323
DV3	MgSO ₄ , 7 H ₂ O	25	1
DV4	Na ₂ CO ₃	21	0,95
DV5	Na ₂ SiO ₃ , 5 H ₂ O	21,2	2
DV6	Fe-EDTA solution	-	10
DV7	Trace elements solution	-	0,1
DV8	vitamin B ₁₂ solution	0,0001	1
DV9	Biotin solution	0,01	0,05
DV10	Thiamine solution	0,1	1

Complete to 1 liter with milli-Q water.

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

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