

	<b>Instruction</b>	Réf. : <b>IT-09</b>
	<b>Preparation of BOLD medium (BB)</b>	Version : 1 Date : 28/11/2011 Page : 1/2

*Référence : BISCHOFF and BOLD, 1963, CCAP, modified at Thonon les Bains.*

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 solutions stocks :

- BB1 : NaNO<sub>3</sub> at 85 g/L
- BB2 : K<sub>2</sub>HPO<sub>4</sub>, 3H<sub>2</sub>O at 40,62 g/L or (K<sub>2</sub>HPO<sub>4</sub> at 20 g/L)
- BB3 : KH<sub>2</sub>PO<sub>4</sub> at 17,5 g/L
- BB4 : CaCl<sub>2</sub>, 2 H<sub>2</sub>O at 36,8 g/L
- BB5 : MgSO<sub>4</sub>, 7 H<sub>2</sub>O at 25 g/L
- BB6 : NaCl at 2,5 g/L
- BB7 : EDTA-KOH solution (\*)
- BB8 : Solution of FeSO<sub>4</sub>, 7 H<sub>2</sub>O acidified at 4,98 g/L (\*\*)
- BB9 : H<sub>3</sub>BO<sub>3</sub> at 11,42 g/L
- BB10 : Trace elements solution (\*\*\*)

#### (\*) EDTA-KOH solution :

In a 100 ml volumetric flask, weigh 5 g of EDTA (or 5,536 g of Na<sub>2</sub> EDTA, 2 H<sub>2</sub>O) and 3,1 g of KOH.

Then adjust the volume with Milli-Q water.

#### (\*\*) Solution of FeSO<sub>4</sub>, 7 H<sub>2</sub>O acidified :

In a 100 ml volumetric flask, weigh 0,498 g of FeSO<sub>4</sub>, 7 H<sub>2</sub>O and transfer 0,1 ml of concentrated H<sub>2</sub>SO<sub>4</sub>.

Then adjust the volume with Milli-Q water.

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**Preparation of BOLD medium (BB)**

(\*\*\*) Trace elements solution :

In a 500 ml volumetric flask, weigh :

- 4,41 g of ZnSO<sub>4</sub>, 7 H<sub>2</sub>O
- 0,355g of MoO<sub>3</sub>
- 0,245g of Co(NO<sub>3</sub>)<sub>2</sub>, 6 H<sub>2</sub>O
- 0,72 g of MnCl<sub>2</sub>, 4 H<sub>2</sub>O
- 0,785g of CuSO<sub>4</sub>, 5 H<sub>2</sub>O

Then adjust the volume with Milli-Q water.

**Preparation of one liter of BB culture medium**

N <sub>o</sub> of stock solution	Name of stock solution	Concentration of stock solution(g/L)	Volume of stock solution (mL)
BB1	NaNO <sub>3</sub>	85	2,94
BB2	K <sub>2</sub> HPO <sub>4</sub> , 3 H <sub>2</sub> O	40,62	2,40
BB3	KH <sub>2</sub> PO <sub>4</sub>	17,5	10
BB4	CaCl <sub>2</sub> , 2 H <sub>2</sub> O	36,8	0,679
BB5	MgSO <sub>4</sub> , 7 H <sub>2</sub> O	25	3
BB6	NaCl	2,5	10
BB7	EDTA-KOH solution	-	1
BB8	Solution of FeSO <sub>4</sub> , 7 H <sub>2</sub> O acidified	4,98	1
BB9	H <sub>3</sub> BO <sub>3</sub>	11,42	1
BB10	trace elements solution	-	1

Complete to 1 liter with fresh MilliQ water.

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

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