2016-07-14

## **ESTdark Medium**

Pers. comm. Mix Modified by MZCH

## **Composition of ESTdark Medium.**

	stock so (1		volume of stock for 1 L nutrient solution	final concentration
	[mmol/L]	[g/L]	[mL]	[mmol/L]
KNO <sub>3</sub>	1000	101.11	2	2
CaCl <sub>2</sub> ·6H <sub>2</sub> O	22.82	5	0.1	0.002
FeCl <sub>3</sub>	6.16	1	1.5	0.009
MgSO <sub>4</sub> ·7H <sub>2</sub> O	40.57	10	1	0.04
$(NH_4)_2HPO_4$	151.45	20	2	0.303
Na₂EDTA·2H₂O (Titriplex® III)	26.86	10	1	0.027
micronutrient solution <sup>1</sup>			2.5	
soil extract <sup>2</sup>			300	
NP-H <sub>2</sub> O <sup>3</sup>			689.9	

Adjust **pH** to **6**.

<sup>&</sup>lt;sup>1</sup>micronutrient solution stock (400x, 1 L)

	stock [mmol/L]	stock [g/L]	
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.016	0.004	
$Co(NO_3)_2 \cdot 6H_2O$	0.137	0.04	
H <sub>3</sub> BO <sub>3</sub>	6.47	0.4	
MnSO <sub>4</sub> ·4H <sub>2</sub> O	0.36	0.08	
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.165	0.04	
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	1.39	0.4	
ddH <sub>2</sub> O <sup>4</sup> , ad 1 L			

## <sup>2</sup>preparation of soil extract:

Weigh 50 g of beech forest soil in a 1 L Erlenmeyer flask. Add 625 mL of dest.  $H_2O$ . Heat the soil solution and keep at 100 °C for 5 minutes. Place a piece of pleated filter paper on top of a second 1 L Erlenmeyer flask and fill a spatula tip of  $CaCO_3$  on the filter. Filter the soil extract (if necessary overnight) and then stir for 15 min. The extract is then centrifuged for 15 minutes at 2500 g and 20 °C. Transfer and aliquot the supernatant into 50 mL Falcon tubes. Store at -20°C.

The volume of the added soil extract should be adapted to the nutrient requirements of the cultured strains.

<sup>3</sup>NP-H<sub>2</sub>O nanopure water, Purelab Pulse (ELGA Lab water, Celle, Germany)

<sup>4</sup>ddH<sub>2</sub>O double distilled water

## Reference

Pers. Comm. Marianne Mix (Univ. Hamburg).