

2016-07-14

## Wd Medium

Waris (1953)

Modified by MZCH

### Composition of Wd Medium.

	stock solutions (1000x, 1 L)		volume of stock for 1 L nutrient solution [mL]	final concentration [mmol/L]
	[mmol/L]	[g/L]		
<b>KNO<sub>3</sub></b>	1000	101.11	1	1
<b>MgSO<sub>4</sub>·7H<sub>2</sub>O</b>	81.14	20	1	0.081
<b>(NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub></b>	151.46	20	1	0.151
<b>CaSO<sub>4</sub> (saturated sol.)<sup>a)</sup></b>		172	1	
<b>micronutrient solution<sup>1</sup></b>			1	
<b>soil extract<sup>2</sup></b>			50	
<b>FeEDTA solution<sup>3</sup></b>			1	
<b>vitamin solution<sup>4</sup></b>			1	
<b>dd-H<sub>2</sub>O<sup>5</sup></b>			943	

Adjust pH to 6 (HCl)

<sup>a)</sup>modified by MZCH

<sup>1</sup>micronutrient solution (1000x, 1 L):

	stock [mmol/L]	stock [g/L]
<b>CoCl<sub>2</sub>·6H<sub>2</sub>O</b>	0.0168	0.004
<b>H<sub>3</sub>BO<sub>3</sub></b>	18.437	1.14
<b>MnCl<sub>2</sub>·4H<sub>2</sub>O</b>	0.728	0.144
<b>Na<sub>2</sub>EDTA·2H<sub>2</sub>O (Titriplex® III)</b>	8.06	3
<b>ZnSO<sub>4</sub>·7H<sub>2</sub>O</b>	0.073	0.021
<b>ddH<sub>2</sub>O<sup>5</sup>, ad 1 L</b>		

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

Weigh 50 g of unfertilized acre soil in a 1 L Erlenmeyer flask. Add 625 mL of dest. H<sub>2</sub>O. 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<sub>3</sub> 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>FeEDTA solution (stock, 100x):

Add 0.52 g **EDTA (Titrplex® II)** and 0.5 g **FeSO<sub>4</sub>·7H<sub>2</sub>O** to 5.4 mL of **1 M KOH** solution, ad 100 mL ddH<sub>2</sub>O<sup>5</sup>

## <sup>4</sup>vitamin stock solution (1000x, 1 L):

	stock [mmol/L]	stock [g/L]
<b>thiamine HCl (B1)</b>	0.296	0.1
<b>biotin (H)</b>	0.0041	0.001
<b>cyanocobalamin (B12)</b>	0.00015	0.0002
<b>nicotinamide</b>	0.0008	0.0001
<b>ddH<sub>2</sub>O<sup>5</sup>, ad 1 L</b>		

Add vitamin solution to the autoclaved and cooled-down medium via sterile filtration.

<sup>5</sup>ddH<sub>2</sub>O double distilled water

## Reference

Waris, H. (1953) The significance for algae of chelating substances in the nutrient solutions. *Physiol. Plant.* **6**, 538-43.