

## WEES Medium

Kies, L. (1967): Über Zellteilung und Zygotenbildung bei *Roya obtusa* (Bréb.) West et West. - Mitteilungen des Staatsinstituts für Allgemeine Botanik Hamburg, Vol. 12: p. 35-42.

Engels, M. (1995): Liste der Sammlung von Conjugaten-Kulturen (SVCK) am Institut für Allgemeine Botanik der Universität Hamburg: Mitteilungen des Institut für Allgemeine Botanik der Universität Hamburg, Vol. 25: p. 65-98.

For 1000 mL final culture medium add the following quantities (Volume) of stock solutions (SL) prepared at the given concentrations to 850 mL dd-H<sub>2</sub>O. Add **one component after the other until each one has completely mixed** and finally fill up to 1000 mL.

All stock solutions can be stored unsterilised at 4 °C. Soil extract should be autoclaved twice on consecutive days and stored at +4 °C. Store vitamin mix (SL 8) at -20 °C.

Stock Solution (SL)	Volume	Component	Concentration in SL	Conc. in final Medium
SL 1	1 mL	KNO <sub>3</sub>	10.0 g · 100 mL <sup>-1</sup>	9.89 · 10 <sup>-4</sup> M
SL 2	1 mL	MgSO <sub>4</sub> · 7H <sub>2</sub> O	2.0 g · 100 mL <sup>-1</sup>	8.11 · 10 <sup>-5</sup> M
SL 3	1 mL	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	2.0 g · 100 mL <sup>-1</sup>	1.51 · 10 <sup>-4</sup> M
SL 4	1 mL	CaSO <sub>4</sub>	saturated solution	
SL 5	1 mL	trace elements	see below	
SL 6	1 mL	Fe-solution	see below	
SL 7	100 mL	soil extract	see below	
SL 8	1 mL	vitamin mix	see below	add aseptically after medium (SL 1 to 7) has been autoclaved

**Adjust medium to final pH of 5.5 or as desired with 0.1n HCl and autoclave at 121 °C for 30 min.**

SL 5	Na <sub>2</sub> EDTA · 2H <sub>2</sub> O (Titriplex III)	0.3 g · 100 mL <sup>-1</sup>	8.05 · 10 <sup>-6</sup> M
Trace elements solution without Fe	H <sub>3</sub> BO <sub>3</sub>	0.114 g · 100 mL <sup>-1</sup>	1.84 · 10 <sup>-5</sup> M
	MnCl <sub>2</sub> · 4H <sub>2</sub> O	14.4 mg · 100 mL <sup>-1</sup>	7.27 · 10 <sup>-7</sup> M
	ZnSO <sub>4</sub> · 7H <sub>2</sub> O	2.1 mg · 100 mL <sup>-1</sup>	7.30 · 10 <sup>-8</sup> M
	CoCl <sub>2</sub> · 6H <sub>2</sub> O	0.4 mg · 100 mL <sup>-1</sup>	1.68 · 10 <sup>-8</sup> M

Combine all trace elements in one SL of 100 mL. Dissolve each component completely one after the other. It may need autoclaving to dissolve. Trace elements solution should **not** be stored in glass containers, but instead in teflon or polycarbonate containers to prevent adsorption of metals to container surface.

SL 6	EDTA ( <b>not</b> Na-salt, Titriplex II)	0.52 g · 100 mL <sup>-1</sup>	1.77 · 10 <sup>-5</sup> M
Fe-EDTA solution	FeSO <sub>4</sub> · 7H <sub>2</sub> O	0.50 g · 100 mL <sup>-1</sup>	1.79 · 10 <sup>-5</sup> M
	1n-KOH	5.4 mL · 100 mL <sup>-1</sup>	5.40 · 10 <sup>-5</sup> M

Prepare SL 6 in a final volume of 100 mL. Dissolve each component completely one after the other. This solution should **not** be stored in glass containers, but instead in teflon or polycarbonate containers to prevent adsorption of Fe to container surface.

SL 7 (soil extract)	garden soil (unfertilised)	10 g · 125 mL <sup>-1</sup>
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Boil 10 g soil (e.g. unfertilised garden soil) in 125 mL distilled water for 5 minutes, let sediment, decant supernatant and centrifuge (15 min. at 5500 rpm), then filter through 1.2-3 µm filter until clear. Tyndallize (important to kill fungal spores!): heat the extract to 100 °C for 15-30 min., then rapidly cool to room temperature and let stand for 24 h; repeat this two more times on consecutive days. Finish by one autolave cycle (121 °C for 30 min.). Store at +4 °C.

SL 8	Vit. B <sub>1</sub> (Thiamin-HCl)	100.0 mg · L <sup>-1</sup>	2.97 · 10 <sup>-8</sup> M
Vitamin mix	Vit. H (Biotin)	1.0 mg · L <sup>-1</sup>	4.09 · 10 <sup>-9</sup> M
	Vit. B <sub>12</sub> (Cobalamin)	0.2 mg · L <sup>-1</sup>	1.20 · 10 <sup>-10</sup> M
	Vit. B <sub>3</sub> (Niacin)	0.1 mg · L <sup>-1</sup>	8.10 · 10 <sup>-10</sup> M

Prepare SL 8 in a final volume of 1 L. Dissolve each component completely one after the other. For storage acidify to a pH of 4.5-5.0 and autoclave, or dispense aseptically through 0.2 µm sterile filters in plastic containers (reaction vials, cryovials, polycarbonate tubes) in 1 mL aliquots and **add aseptically to prepared medium after autoclaving and cooling**. Store at -20 °C.

For stock cultures on agar slants add 1.0-1.3 % Agar (e.g. purified high strength, 1000 g · cm<sup>-2</sup>) to prepared medium before autoclaving.