

## BacTest ES-Fleisch Medium (Basal Medium ES + Beef Extract)

This medium is used to check stock cultures for fungal and bacterial contaminations. It is prepared as a liquid medium. Autoclaved, standard clear glass culture tubes are filled with 5 mL of this medium aseptically, and these are stored at 4 °C until used. These controls should be performed regularly (e.g. every 1 or 2 years depending on serial transfer intervals of stock cultures) to detect contaminations, and thus, maintain a high quality of the stock cultures.

Taken the high diversity of fungi and bacteria and their specific growth requirements into account, not all of these unwanted organisms might grow in this medium. As detection is done with the naked eye by comparison to a zero-control, it is necessary that a sufficient number of contaminants has grown within a reasonable time (usually within 1-2 weeks) resulting in a cloudiness in positive control tubes. Microscopical observation might be necessary in doubtful cases or a prolonged incubation. Also, additional media with different nutrients and pH values might have to be used.

For 1000 mL final culture medium add the following quantities (Volume) of stock solutions (SL) prepared at the given concentrations to 905 mL distilled water. Add **one component after the other until each one has completely mixed**. All stock solutions can be stored unsterilised at 4 °C.

Stock Solution (SL)	Volume	Component	Concentration in SL	Conc. in final Medium
SL 1	20 mL	KNO <sub>3</sub>	1.0 g · 100 mL <sup>-1</sup>	1.98 mM
SL 2	20 mL	K <sub>2</sub> HPO <sub>4</sub>	0.1 g · 100 mL <sup>-1</sup>	0.11 mM
SL 3	20 mL	MgSO <sub>4</sub> · 7H <sub>2</sub> O	0.1 g · 100 mL <sup>-1</sup>	0.08 mM
SL 4	30 mL	soil extract (see below)	-	-
SL 5	5 mL	micronutrient solution (see below)	-	-
SL 6	1 g	Beef Extract	-	0.1 %
	905 mL	distilled water		

### Preparation of the soil extract (SL 4)

Boil 50 g of soil (garden or deciduous forest soil; no fertilisers nor pesticides) in 500 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.

### Preparation of the micronutrient solution (SL 5)

For 1000 mL final micronutrient solution prepare the following individual stock solutions of each micronutrient in 100 mL of distilled water and add the volumes / amounts indicated to the final volume of distilled water as indicated for SL 5a and SL 5b). Autoclave SL 5a and SL 5b separately to avoid precipitation of salts, let cool and combine both to final micronutrient solution SL 5 to prepare the final BacTest ES-Fleisch Medium. 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 5	Volume	Component	individual SL Concentration	Conc. Final Medium
SL 5a	1 mL	ZnSO <sub>4</sub> · 7H <sub>2</sub> O	0.1 g · 100 mL <sup>-1</sup>	1.74 · 10 <sup>-8</sup> M
(total volume = 900 mL)	2 mL	MnSO <sub>4</sub> · 4H <sub>2</sub> O <sup>1</sup> (!)	0.1 g · 100 mL <sup>-1</sup>	4.48 · 10 <sup>-8</sup> M
	5 mL	H <sub>3</sub> BO <sub>3</sub>	0.2 g · 100 mL <sup>-1</sup>	8.09 · 10 <sup>-7</sup> M
	5 mL	Co(NO <sub>3</sub> ) <sub>2</sub> · 6H <sub>2</sub> O	0.02 g · 100 mL <sup>-1</sup>	1.72 · 10 <sup>-8</sup> M
	5 mL	Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O	0.02 g · 100 mL <sup>-1</sup>	2.07 · 10 <sup>-8</sup> M
	1 mL	CuSO <sub>4</sub> · 5H <sub>2</sub> O	0.0005 g · 100 mL <sup>-1</sup>	1.00 · 10 <sup>-10</sup> M
	0.4 g	Na <sub>2</sub> EDTA (Titriplex III)		-
	881 mL	distilled water		
SL 5b	0.7 g	FeSO <sub>4</sub> · 7H <sub>2</sub> O		1.26 · 10 <sup>-5</sup> M
(total volume = 100 mL)	0.4 g	Na <sub>2</sub> EDTA (Titriplex III)		-
	100 mL	distilled water		

This results in the the BacTest ES-Fleisch Medium. The pH-value will be about 6.5.

<sup>1</sup> at CCCryo 0.1 g MnSO<sub>4</sub> · 4H<sub>2</sub>O is replaced by 0.0755 g MnSO<sub>4</sub> · H<sub>2</sub>O