

PROTOCOL: Preparation of growth media

Lysogeny broth medium (LB)

Material and chemicals:

For 1 l of LB medium:

Yeast extract	5 g	<input type="checkbox"/>
Tryptone	10 g	<input type="checkbox"/>
NaCl	10 g	<input type="checkbox"/>

Mix components in 800 ml distilled H₂O and once all chemicals are dissolved, add dH₂O to the final volume of 1 liter.

pH of the medium should be around 7. If not, adjust it by adding an appropriate amount of NaOH (medium is usually more acidic). Then autoclave for 23 min, 121 °C, 101,3 kPa.

Notes:

- For 1 l of LB medium, we added 0,5 ml of 2M NaOH to achieve pH 7.
- We used this medium for growing *Escherichia coli* and also for *Bacillus subtilis*.
- This medium is suitable for preparation of overnight culture and e.g. preparation of dilution series and so on.
- The medium has to have a brightly yellow color and has to be completely clear.
- If you smell a sour odor after opening the media, your media is probably contaminated. You have to prepare a new one.
- You can store flasks with this sterile media at room temperature. But after opening the flask and using only a fraction of the medium, it is better to store the flask at 4°C.
- Open the flask only in a flow box and do not forget to tan the throat of the flask upon the flame.

Lysogeny broth agar (LB agar)

Material and chemicals:

For 250 ml of LB agar:

Yeast extract	1.25 g	<input type="checkbox"/>
Tryptone	2.5 g	<input type="checkbox"/>
NaCl	2.5 g	<input type="checkbox"/>
Bacterial agar	3.75 g	<input type="checkbox"/>

Mix components in 200 ml distilled H₂O and once all chemicals are dissolved, add dH₂O to the final volume of 250 ml.

pH of the medium should be around 7. If not, adjust it by adding an appropriate amount of NaOH (medium is usually more acidic). Then autoclave for 23 min, 121 °C, 101,3 kPa.

Notes:

- For 0,25 l of LB agar, we added 0,2-0,25 ml of 2M NaOH to achieve pH 7.
- For 0,25 l of LB agar, always use a bigger flask - at least 0,5 l.
- This growing media is appropriate for growing media on the top of the plate for *Escherichia coli* and *Bacillus subtilis* and many others.
- You can store the LB agar at room temperature. When preparing plates, boil it in the microwave and then pour plates.
- You can add antibiotics to the medium before pouring plates, but make sure that the medium has around 50 °C, not more.
- You must never autoclave LB agar with antibiotics!
- Store poured plates at 4 °C.

Used antibiotics:

ATB	Usage	Stock concentration	Diluted in	Final concentration in medium	How much to the 250 ml of LBA
Ampicillin	selection of <i>E. coli</i> transformants	150 mg/ml	Milli-Q H ₂ O	150 µg/ml	250 µl
Lincomycin	MLS, selection of <i>B. subtilis</i> transformants with pDG1664	25 mg/ml	Milli-Q H ₂ O	12.5 µg/ml	125 µl
Erythromycin	MLS, selection of <i>B. subtilis</i> transformants with pDG1664	10 mg/ml	ethanol	0.5 µg/ml	12.5 µl
Spectinomycin	Negative selection for construct insertion	50 mg/ml	ethanol	100 µg/ml	500 µl
Chloramphenicol	selection of <i>B. subtilis</i> transformants with pDG3661	50 mg/ml	ethanol	5 µg/ml	25 µl

- The stocks of antibiotics were stored at -20 °C.