

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"/>
dH ₂ O	1 l	<input type="checkbox"/>
mix and autoclave (15 min, 121 °C)		<input type="checkbox"/>

Notes:

- 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 sour odor after opening the media, your media is probably contaminated. You have to prepare a new one.
- You can store flask 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.
- pH should be around 7 (but in this media is not pH measurement necessary)

Lysogeny broth agar (LB agar)

Material and chemicals:

For 1 l of LB medium:

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"/>
dH ₂ O	250 ml	<input type="checkbox"/>
mix and autoclave (15 min, 120 °C)		<input type="checkbox"/>

Notes:

- This growing media is appropriate for growing media on the top of the plate for *Escherichia coli* and *Bacillus subtilis* and lots of others..
- You have to sterilize the medium before pouring the plates.
- After preparing, it is better to pour the media in the plates immediately (it smells horrible if the medium is contaminated and you have to boil it in the microwave for pouring out - it is much easier to throw out some contaminated plates).
- But you can also store it in the flasks and boil in the microwave before pouring on the plates.
- Plates should be stored in 4 °C.
- You can add antibiotics to the medium before pouring plates but ensure that medium has around 45 °C and not more.
- You do not have to autoclave LB with antibiotics!
- Do not autoclave in the higher volume than 250 ml in the 1 l flask.

We usually work with this antibiotics:

ATB	Usage	Stock concentration	Diluted in	Final concentration in media	How much to the 250 ml of LBA
Ampicillin	selection of transformants of <i>Escherichia coli</i>	150 mg/ml	H ₂ O	100 µg/ml	167 µl
Chloramfenicol	Selection of transformants of <i>Bacillus subtilis</i> with pDG3661	50 mg/ml	ethanol	5 µg/ml	25 µl

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