

PROTOCOL: AmyE test (Starch hydrolysis test)

Material and chemicals:

Solid 1.6% LB agar plates
 Bacterial agar
 Starch
 dH₂O

To dissolve starch, you have to bring the solution to the boiling point. Boiling also sterilizes the media - boil at least 15 minutes.

Bacterial agar	1%	<input type="checkbox"/>
Starch	1%	<input type="checkbox"/>
dH ₂ O	100 ml	<input type="checkbox"/>
Boil with mixing until the solution is clear (dark yellow)		<input type="checkbox"/>

Notes:

- You can store this solution in a closed flask at room temperature. Boil in the microwave before next use.
- Prepare a higher amount of media - you never know how many times you will repeat the test. 100 ml of the media is for preparation of 20 plates.

Workflow:

1. Weight appropriate amount of agar and starch in a glass flask. Pour in the distilled water and mix properly.
2. Put a magnetic mixing stick inside and put the flask at the magnetic hotplate. Turn the mixing and heating on.
3. Bring to the boil and mix until the solution becomes clear. Do not forget to take out the mixing stick.
4. Let the solution cool down to approximately 45 °C and pour 5 ml of this solution on the top of LB agar which you prepared beforehand (if you want to use antibiotics and you do not have antibiotics in the LBA which were done before, you can also put the antibiotic to this thin starch layer). Let the layer solidify.
5. Steak bacteria onto this special plate. Do not forget to prepare control plates (e.g. *Bacillus subtilis* without integrated gene - count with the fact that these bacteria are not usually resistant to antibiotics, so use a plate without antibiotics). Incubate at 37 °C overnight.
6. The next day in the morning, put a few iodine crystals in the “empty” half of the Petri dish and cover it with the other half that contains the agar and bacteria (agar layer is on the top, the crystals lay straight on the plastic or glass) so the iodine vapor interacts with the starch in the agar.
7. In a few minutes, the starch starts to turn dark blue. Do not forget to take a picture of your plates because the starch will turn lighter after a certain amount of time.