

Basics of creating media

1. Before you start working in the Lab put on the lab coat and gloves;
2. Read attentively an instruction on the box of dry media which you are going to prepare.
3. According to instruction (for example for Brain Heart Agar) take one liter of desterilized water and pour it in the glass fireproof Flask. (The glass fireproof Flask is graduated, gaps tells about its capacity);
4. Place measurement plate on electro balance to find out its weight.
5. Weight 52 gr media powder;
6. Place powder in flask with desterilized water;
7. Place the flask on electro heater and by glass stick stir until dry powder dissolves. The resulted liquid should be boiled 2-3 min.
8. Take prepared media from electro heater.
9. Measure PH of media using paper indicator.
10. Take one PH paper on the one end and place it half into the prepared liquid, damp it and take out.
11. Take PH box in one hand and in other hand PH colored paper.
12. Compare colored paper indicator to the colors mentioned on the PH box;
13. Colors mentioned on the box matches numbers. (it is different for different microorganism species).
14. The optimal PH for most bacterial species is $7,2 \pm 0,2$.
15. Add NAOH or HCl if the PH of media is not satisfied according to alkali or acid reaction you want to get until getting the optimal PH.
16. Pour prepared media in the sterail gapped glass boteles (In the gapped glass bottles with capacity 250ml we put 150ml media and in the 500ml bottles - 300ml);
17. Put paper ribbon on glass bottles and label with marker – nomination, percentage, the date of preparing.
18. Put the bottles in the autoclave (15mn on 121°C);
19. After time out and switch of autoclave.
20. Wait before autoclave become cooler and take out media,
21. Wait until it's become solid and keep in cool place (refrigerator) + $4-5^{\circ}\text{C}$;
22. Switch on the laminar box use button on the control panel of box.
23. Put down the front glass.
24. Switch on the UV light for 30 min use another button on the control panel of box.
25. Afterwards switch off UV light.
26. Turn on air source and the light by help of another button;
27. Melt the media in microwave oven;
28. Put sterile plastic or glass Petri dishes and melted media in the laminar box.
29. Before working take a place in front of box
30. Carefully unpack melted media bottle from foil paper and cover.

31. Take bottle in left hand, with right hand take up half the Petri dishes cover and pour 30ml of media, after quickly put down the cover in initial condition;
32. Do the same until bottle is empty.
33. Media on Petri dishes become solid and should be kept with cover down to avoid accommodation of big amount of condensate on the media surface.
34. Label Petri dishes by marker – type of media and date.
35. Keep Petri dishes with media in the refrigerator on + 4-5°C.