# Bio Bharati Life Science Pvt. Ltd.

## Plasmid DNA isolation

**Cat# BB-MTK020S (5 reactions)**
**Cat# BB-MTK020 (10 reactions)**

**Introduction:**

- Plasmids are self-replicating, double stranded, circular DNA molecules that are maintained in bacteria as independent extra chromosomal entities.
- The size of plasmid ranges from 1 Kbp to 500 Kbp

**Principle:** Bacterial host grow in LB broth medium at recommended Ampicillin concentration. Ampicillin helps to maximize the copy no. of plasmid.

Solution I contain EDTA which act as a chelating agent to chelate the $Mg^{++}$ in the cell wall to result weakening of cell walls. The weakened cell walls are then lysed by NaOH & SDS present in Solution II.

Solution II also brings about the precipitation of proteins, chromosomal & plasmid DNA of bacteria. Keep the Solution II in room temperature to avoid precipitation of SDS. If it precipitates then it should be kept at $37^0$C water bath / incubator until it dissolves.

Solution III contains Potassium Acetate which acts as a renaturing agent of denatured plasmids by creating alkaline environment.

Denatured chromosomal DNA & proteins will be removed by centrifugation. The supernatant has the plasmid which is concentrated by Isopropanol (Solution IV) and washed by Ethanol (Solution V).

DNA isolation involves the following steps:

1. Bacterial cell harvesting & cell lysis.
2. Denaturation of proteins & cell debris.
3. Renaturation & precipitation of plasmid.
4. Purification of plasmid.

**Materials provided & storage condition:**

| Sl No | Items | Quantity for 10 experiments | Store at |
|---|---|---|---|
| 1. | Solution I | 8 ml | $4^0$C |
| 2. | Solution II | 8 ml | RT |
| 3. | Solution III | 8 ml | $4^0$C |
| 4. | Solution IV | 8 ml | RT |
| 5. | Solution V (Wash Buffer) | 8 ml | $4^0$C |
| 6. | Ampicillin (100 mg/ml) | 600 µl | $-20^0$C |
| 7. | Host | (DH5alpha with pUC18) | $4^0$C |
| 8. | Control DNA ( 100 ng/λ) | 60µl (single Digested pUC18) | $-20^0$C |
| 9. | RNase (10 mg/ml) | 20 µl | $-20^0$C |
| 10. | Elution Buffer | 2 ml | 4 C |
| 11. | Agar | 2gm | RT |
| 12. | LB medium | 5gm | RT |
| 13. | Agarose | 2gm | RT |
| 14. | 6x DNA Dye | 50µl | RT |
| 15. | Ethidium Bromide | 25µl | RT |
| 16. | 50 x TAE | 30 ml | $4^0$C |
| 17. | DNA Marker | 50µl | $-20^0$C |
| 18. | 1.5 ml empty Vial | 40 nos | RT |

**Protocol:**

**Day 1:**

Inoculate a loop full culture into 2-5 ml of LB medium containing Ampicillin at a concentration of 100µg/ml & incubate at $37^0$C for overnight in a shaking incubator.

**Day 2:**

- Aliquot 1.5 ml of overnight bacterial culture into provided fresh autoclaved 1.5ml centrifuge tube.
- Centrifuge at 6000rpm for 10 minutes.
- Discard the supernatant & drain the remaining supernatant by keeping the vial inverted on a blotting paper.
- Now, dissolve the pellet with 200µl Solution I. No clumps of cells should be seen after resuspension of the pellet. After dissolving the pellet keep the centrifuge tube at room temperature for 4-5 mins.
- Add 400 µl Solution II & invert the tubes 4-6 times gently for proper mixing, do not allow the lysis reaction to proceed more than 5mins. *Vortexing is strictly prohibited in this step as it will result in shearing of DNA.*

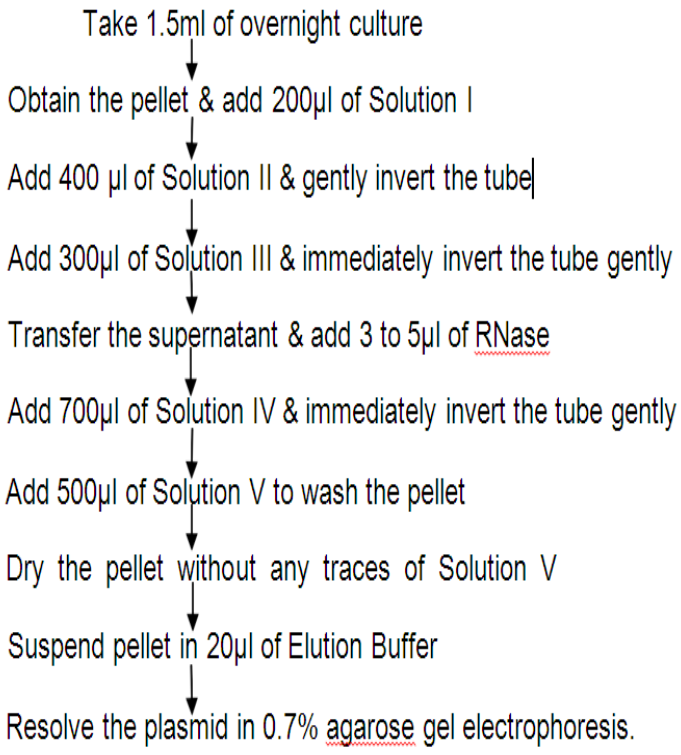
**[It is strongly recommended to keep the Solution II in room temperature to avoid precipitation of SDS. If it precipitates then it should be kept it at $37^0$C waterbath / incubator until it dissolves.]**

- Add 300 µl of Solution III. To avoid localized precipitation, mix the solution gently but thoroughly immediately after addition of Solution III. The solution will become cloudy.
- Centrifuge at 13500 rpm for 20 min at $4^0$C. After centrifugation, the chromosomal DNA along with SDS, bacterial proteins & debris, will pellet.
- Collect the supernatant into another microcentrifuge tube (autoclaved). Add 1-3µl of RNase (for cleavage of RNA) into the collected supernatant containing plasmid DNA & keep it at $37^0$C for 1-2hrs.
- Add 0.7 volume (700µl of 1ml supernatant) of Solution IV into the micro centrifuge tube & invert the tube for 7 to 10 times for proper mixing of solution IV with supernatant.
- Centrifuge the tube at 13500rpm for 20mins at $4^0$C. Discard the supernatant. Minute & clear white deposits can be seen in the side of the microcentrifuge tube.
- Add 500µl of Solution V into the microcentrifuge tube & centrifuge at 13500 rpm for 10mins at $4^0$C.
- Dry the tube containing the pellet by inverting the tube on a paper towel at room temperature till there are no traces of Solution V.
- Add 20-30 µl of EB (Elution Buffer) & gently suspend the pellet to dissolve the plasmid DNA. Resuspend the DNA gently without vortexing or repeat pipetting to avoid the shearing of DNA.
- Run 0.7% agarose gel electrophoresis to observe the DNA bands using UV transilluminator. Compare the newly prepared Plasmid DNA bands with the control DNA provided (5µl loading).

**Preparation of 0.7% agarose gel electrophoresis:**

- Prepare 0.7% agarose solution in 50x TAE buffer provided (e.g. for 50 ml of agarose gel add 0.35gm of agarose, 1 ml of TAE & 49 ml of sterile water).
- Boil until the agarose completely dissolves.
- After boiling allow the solution to cool down to around 37-40$^0$C.
- Add 2.5 µl of Ethidium Bromide (very carefully) & mix properly.
- Seal the gel casting tray on two sides; place the comb in the gel casting tray in appropriate position.
- Pour the agarose mixture slowly (to avoid generation of any air bubble) in the tray containing comb.
- Let the agarose solidify in the tray. Then remove the seal from the two sides without disturbing the gel.
- Next keeps the gel tray in the tank containing 1X TAE buffer. Keep the wells of the gel in the cathode (Negative) side. The buffer level in the tank should be maintained above the gel tray.
- Lift the comb from the gel gently to avoid damaging of wells; the gel is now ready for loading.
- Connect the cords of electrophoresis tank with the power pack before loading the samples.
- To prepare samples for electrophoresis add 3-5µl of 6X gel loading dye into the sample & mix well by pipetting or pulse centrifugation. Load 20µl of sample into the well.
- Then switch on the power pack & adjust the voltage between 60V to 100V.
- Continue the electrophoresis until the dye front reaches to1/3$^{rd}$ or above of the gel.
- Observe the DNA bands using UV Transilluminator.

**Flow chart:**




**Result and Interpritation:**

The plasmid DNA isolated by alkaline lysis method is separated by agarose gel electrophoresis & visualized with UV Transilluminator which shows circular, linear, supercoiled forms of plasmid DNA.

*Adapted from internet*