Lot.
Ref. SB0071-74

MANUAL

Expiry date: 2 years
-Only for research use-
-Store at room temperature-
-To be used by a technical person-

DNA ISOLATION KIT

This kit can be used to isolate the DNA from the blood samples (including blood spots on filter paper / normal paper / tissue paper / a piece of cloth), plasmids, bacterial colonies, cell lines, cell cultures, serum (plasma) as well as tissue samples. It can be used to isolate from sperms, spinal fluids, tissue, tissue pieces, mouse tail, ear punches, buccal swabs, very little bloodspots as well as bloodstained clothes and wood.

This kit is quick and effective. It does not need any expensive instruments.
Moreover, all components **can be kept at room temperature**.

Components:
Solution A: It contains sodium hydroxide. Use protective clothes!
Solution B: Buffer
Solution C: Solution

Instruments needed:
Heat Block
Pipettor and Pipettes
Tubes

Solution Z Preparation:
This solution must be prepared **freshly before use (very important)**. Calculate how much solution is needed for the isolations. Here is an example of 10 isolations. Add 450 µl of solution C to one tube and add 50 µl of solution A to it gently (the dilution rate is 1:10; 9 parts of solution C and one part of solution A). This is called Solution Z.

Procedure:
a) Isolation from blood samples:
1. Cut the blood spot filter paper (2-3 mm) or cut the cotton swab (2-3 mm) and put this in one 1.5 ml tube at the bottom. Usually it is sufficient to cut 2-3 mm sample. Do not put many pieces of your probe as this method is very sensitive. User may need some experience regarding the amount of the probe to be used. In case user wants to use the liquid form of blood, 20-30 µl will be sufficient. (It is better to put the blood on filter paper and isolate in some cases, but liquid blood functions well too!).

2. Add 100 µl of **freshly prepared solution Z** to your 1.5ml tube containing pieces of probe.

3. Keep this tube at 88 °C for 7 minutes in heating block.

4. Remove the tube from heating block and add 100 µl of solution B to each tube. After Adding solution B, vortex the sample immediately for 5-10 seconds.

5. Add 200 µl of solution C to each tube.

6. Store the tubes at 4 °C for immediately use or -20 °C for further use. It has shown that in some cases, one can get better DNA isolation results after storing for 4-6 hours at 4°C.

7. Use the supernatant as source of DNA (Do not vortex the sample again!). Usually 1µl or 2 µl of this in the PCR. Sometimes, it may be highly concentrated; there may be need of dilution.

Tip: Usually one can use the supernatant immediately, but user has to make experience that it is better to keep this overnight at 4 °C. The supernatants can **be stored at -20 °C** for further use.

b) Isolation from mouse tail or ear or tissue pieces:

1. Cut the tail / ear / tissue (3-4 mm) with clean scissor (scissor must be cleaned with distilled water and with disinfectant before use to avoid the contamination and dried the scissors should be used) and put it in 1.5 ml reaction tube

2. Add 100 µl of **freshly prepared solution Z** to 1.5 ml tube containing probe.

3. Keep this tube at 88 °C for 20 minutes **(in case of tissue only 8 minutes)** in heating block. Do 5 times vortexing during this period.

4. Now remove the tube from heating block and add 100 µl of solution B to each tube. Add this solution in the middle of tube so that solution does not touch the walls in order to avoid loss of solution. Kindly do good vortexing for 5-10 seconds.

5. Add 200 µl of solution C to each tube.

6. Centrifuge the tube for 5 minutes for 11 000 g.

7. Pipette out the 290 µl of supernatant in fresh tube and label this tube. This supernatant contains DNA, which can be used in different applications e.g. conventional PCR, real time PCR etc.

1 -2 µl of this solution is sufficient to run the PCR, but user may need more or even less DNA.

8. It should be stored at 4 °C or at -20 °C for further use.

c) Isolation from buccal swab:

Preparation the solution Z: This must be prepared freshly as this is very important. Calculate how much solution do you need for the isolation of your probes? Add 380 µl of solution C gently to one tube and add to this tube 20 ul of solution A gently to tube (the dilution ratio is 1: 20 i.e. 1 part + 19 parts). Use must use **freshly prepared solution Z as very important!**

To do 20 isolations, take 3800 ul of solution C in a tube. To this tube, add 200 ul of solution A. Dilution ration is 1: 20.

1. Cut the top of buccal swab with clean scissor (Tip: scissor can be cleaned with distilled water and with Ethanol before use) and put it in 1.5 ml reaction tube. This is very important that one scissor should be used for one piece per isolation per tube (clean it before new use!).

2. Add 200 µl of **freshly prepared solution Z** to your 1.5 tube containing probe.

3. Keep the tube at 88 °C for 7 minutes in heating block. During this period, vortex the tube 3-4 times.

4. Now remove the tube from heating block and add 200 µl of solution B to each tube. Add this solution in the middle of tube so that solution does not touch the walls in order to avoid loss of solution. Kindly do good vortexing for 10 seconds.

5. Add 400 ul of tube C to each tube.

6. Centrifuge the tube for 1 minute for 11 000 g.

7. Remove the buccal swab or pipette out the supernatant! Now user has supernatant containing the DNA and can be used in different applications e.g. conventional PCR, real time PCR etc. 1 or 2 µl of this solution is sufficient to run the PCR, but you may need more or even less DNA according to your method. It should be stored at 4 °C or at -20 °C.

D. Isolation from cell cultures (e.g. also viral cell culture suspensions) or plasmids (bacterial colonies):

1.Add 100 µl of **freshly prepared solution Z** to 1.5ml tube. Take a part of colony or complete colony with a loop or wood stick (match box stick may be used in some cases) and add it to freshly prepared Z solution in 1.5 ml tube. In case of cell culture, take 10 ul cell suspension!

2. Keep this tube at 88 °C for 7 minutes in heating block.

3. Remove the tube from heating block and add 100 µl of solution B to each tube. After adding solution B, vortex the sample immediately for 5-10 seconds.

4. Add 200 µl of solution C to each tube. Centrifuge it at 11000 g for 1 minute, if you have added a wooden stick and remove the wooden sticks to discard!

5. Keep the tubes containing solution at 4 °C for 4-10 hours (it is better to keep overnight). For long time use, freeze it at -20 °C. Use the supernatant as source of DNA (Do not vortex the sample again!). Usually 1µl or 2 µl of this in the PCR. Sometimes, it may be highly concentrated; there may be need of dilution.

E. Isolation from serum or plasma:
1. Add 100 µl of **freshly prepared solution Z** to your 1.5ml tube. Add 50-80 µl of serum or plasma to it.

2. Keep this tube at 88 °C for 7 minutes in heating block.

3. Remove the tube from heating block and add 100 µl of solution B to each tube. After adding solution B, vortex the sample immediately for 5-10 seconds.

4. Add 200 µl of solution C to each tube.

5. Store the tubes containing solution at 4 °C for 4-10 hours (it is better to keep overnight). Use the supernatant as source of DNA (Do not vortex the sample again!). Usually 1µl or 2 µl of this in the PCR. Sometimes, it may be highly concentrated; there may be need of dilution.

If you should find any mistakes, please let us know. Thank you.

Suggestion: **This manual has been written specifically for beginners, hence persons with experience in PCR must use their experience to keep each step as small as possible e.g. you should calculate the amount of the needed chemicals, before starting with testing.** **Last update: 26.01.2021** **v1.4**	**Genekam Biotechnology AG** **Duissernstr.65a** **47058 Duisburg** **Germany** **Tel. (+49) 203 / 555858-31,-32,-33** **Fax (+49) 203 / 35 82 99** **http://www.genekam.dee**