Lot.
Ref. SB0071

MANUAL

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

GENEKAM 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) as well as tissue samples. This can be used for human as well as veterinary medicine. It can be used to isolate from sperms, spinal fluids, tissue, tissue pieces, mouse tail, buccal swabs, very little bloodspots, blood-stained clothes and wood.

Kindly note that this kit is quick and effective and does not need any expensive instruments. Moreover all components **can be kept at room temperature**.

Components:
Solution A: It contains sodium hydroxide. Please use goggles during use as well as avoid putting drops of this solution of clothes.
Solution B: Buffer
Solution C: highest grade water

Solution Preparation:
This solution must be made freshly before use, as this is very important. You have to calculate how much solution do you need for your isolations. Add 450 ul of solution C to one tube and add 50 ul of solution A gently (the dilution rate is 1:10; 9 parts of solution C and one part of solution A to this solution). Solution A must be added to solution C as this is very important. This is called Solution Z.

Procedure:
a) Isolation from blood samples:
1. Cut the blood spot filter paper (2 mm) or cut the cotton swab (2 mm) and put this in one 1.5 ml tube at the bottom. Usually it is sufficient to put 1-2 of 2 mm pieces. Do not put more pieces of your probe as this method is very sensitive. You need some experience regarding the amount of the probe needed as this is a very important factor. In case you have more probes, please label 1.5 ml tube with number of name of the probes. In case you are using the liquid form of blood, 20 µl will be sufficient. (It is better to put the blood on filter paper and isolate).

2. Add 100 ul 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 ul of solution B to each tube. After adding solution B you have to vortex the sample immediately for 5-10 seconds).

5. Add 200 ul of solution C.

6. Keep the tubes containing solution at 4 °C for 4-10 hours (it is better to keep overnight).

7. Use the supernant as source of DNA (Do not vortex the sample again !). usually 1ul or 2 ul of this in the PCR. Some times, it may be highly concentrated, there may be need of dilution.

Important point: In case you have blood samples, you can use the cotton swabs to take the probe as this makes the method more effective and easy. In case you have tissue, you need to add only 2mm piece of tissue. Usually one can use the supernant immediately, but we have made experience that it is better to keep this overnigth at 4 °C. For these points, you need experience to be made. The supernants can **be stored at -20 °C** for use for 2-3 months. Once you will be able to use this method, you will be able to save a lot of costs of DNA isolation along with the costs of hardware like pipette tips etc.

b) Isolation from mouse tail:

1. Cut the tail (3 mm) with clean scissor (scissor must be cleaned with distilled water and with Ethanol before use) and put in 1.5 ml reaction tube

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

3. Keep this tube at 88 °C for 20 minutes in heating block. You should do 5 times vortexing during this period.

4. Now remove the tube from heating block and add 100 ul of solution B to each tube. You must 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.

5. Add 100 ul of solution C to each tube.

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

7. Pipette out the 290 ul of supernant in fresh tube and label this tube. This supernant contains the DNA and can be used in different applications e.g. conventional PCR, real time PCR etc. 1 ul of this solution is sufficient to run the PCR, but you may need more or even less DNA according your method.

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

c) Isolation from tissue samples:

One can use tissue samples of human and animal origin like pig, cattle, sheep, goat, chicken etc. Similarly one can use meat pieces also.

1. Cut the tissue (2-3 mm) with clean scissor (scissor must be cleaned with distilled water and with Ethanol before use) and put in 1.5 ml reaction tube. This is very important that one use one piece per isolation per tube. The tissue piece must be very small.

2. Add 100 ul of **freshly prepared solution Z** to your 1.5 tube containing probe. Please do not vortex.

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

4. Now remove the tube from heating block and add 100 ul of solution B to each tube. You must 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.

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

6. Optional step (This step can be omitted also): Centrifuge the tube for 5 minutes for 11 000 g. In case you have omitted this step, you can use supernant, which contains the isolated DNA. During taking the supernant, try to avoid to pipette the tissue piece. The isolated DNA should be kept at 4 °C.

7. Pipette out the 350 ul of supernant in fresh tube without pipetting the debris and label this tube. This supernant contains the DNA and can be used in different applications e.g. conventional PCR, real time PCR etc. 1 or 2 ul of this solution is sufficient to run the PCR, but you may need more or even less DNA according your method. It should be stored at 4 °C or at -20 °C.

d) Isolation from buccal swab:

Preparation solution Z: This must be prepared freshly as this is very important. Kindly calculate how much solution do you need for the isolation of your probes. Add 380 ul of solution C gently to one tube and add to this tube 20 ul of solution gently (the dilution ratio is 1: 20 i.e. 1 part + 19 parts) to do two isolations. Kindly do not add solution A to solution C as solution A must be added to solution C as this is very important. Now you have **freshly prepared solution Z**.

In case you want to do 10 isolations, you have to take 3800 ul of solution in a tube. To this tube, you have to add 200 ul of solution A. Dilution ration is 1: 20.

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

2. Add 200 ul of **freshly prepared solution Z** to your 1.5 tube containing probe. Please vortex this.

3. Keep this tube at 88 °C for 5 minutes in heating block. During this period, vortex the tube 3-4 times.

4. Now remove the tube from heating block and add 200 ul of solution B to each tube. You must 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.

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

7. Remove the buccal swab. Now you have supernant containing the DNA and can be used in different applications e.g. conventional PCR, real time PCR etc. 1 or 2 ul 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.

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: 04-08-2008** **v1.2**	**Genekam Biotechnology AG** **Dammstr. 31-33** **47119 Duisburg** **Germany** **Tel. (+49) 203 / 555858-31,-32,-33** **Fax (+49) 203 / 35 82 99** **anfrage@genekam.de** **http://www.genekam.de**