**Lot.**
**Ref. SB0001-SB0004**

# MANUAL

**Expiry date: 1 year**
**Store at $4^0$C**

GENEKAM DNA ISOLATION KIT

**-Only for research use-**
**-To be used by a technical person-**

## Contents:

- Tube A (lysis buffer 1)
- Tube G (lysis buffer 2)
- Tube K (proteinase K) to be stored at $4^o$ C
- Tube B (washing buffer 1)
- Tube C (washing buffer 2)
- Tube E (elution buffer)
- Mini column
- Collection tubes for mini column ( 2ml with round bottom)
- Collection tubes for mini column ( 1.5 ml with conical bottom) for elution

## Chemicals and equipments needed:

- Molecular ethanol
- Pipettes and Pipette tips
- Heat block
- Centrifuge

## Procedure:

**Standard Step (this can be used with any sample):**

1. Add 300µl of Tube A and 15 µl of Tube K to the sample in the tube.
2. Incubate at 56°C for 20-30 minutes. Add to this 400µl of tube G. Incubate at 70°C for 5 minutes.
3. Add to this 400µl of molecular ethanol and do the vortexing.
4. Take a mini column in one collection tube and add 600µl of above made solution to this mini column.
5. Centrifuge this for one minute at 11000rpm. Discard the filtrated fluid.
6. Add the rest of your remaining fluid in this mini column and repeat the step 5 for centrifuge. Discard the filtrated fluid.
7. Now add 500µl of Tube B to mini column. Repeat the step 5 for centrifugation and discard the filtrated fluid.
8. Add 500µl of Tube C to mini column. Repeat the step 5 for centrifugation and discard the filtrated fluid.
9. Add 200µl of tube C to mini column. Repeat centrifugation for 3 min at 13000rpm and discard the filtrated fluid.
10. Centrifuge the mini column for 1 min at 13000rpm to dry the matrix. Discard the used collection tube.
11. Now put the mini column (filter part) in a new 1.5 ml collection tube.
12. Add 50µl - 100µl of Tube E **(pre-warmed to 70°C)** to the mini column.
13. Now keep this at room temperature for two minutes.
14. Centrifuge this at 13000rpm for one minute.
15. Now you have fluid in the collection tube. This is your isolated DNA. This can be used to conduct different assays. Store your DNA at -20°C for long term application.

**Tips:**

How to do the isolation from the buccal swabs:

1. Cut the head of buccal swabs.
2. Add 300µl of Tube A and 15 µl of Tube K to the sample in the tube.
3. Incubate at 56°C for 20-30 minutes. Add to this 400µl of tube G. Incubate at 70°C for 5 minutes.
4. Add to this 400µl of molecular ethanol and do the vortexing.
5. Take a mini column in one collection tube and add 600µl of above made solution to this mini column.
6. Centrifuge this for one minute at 11000rpm. Discard the filtrated fluid.
7. Add the rest of your remaining fluid in this mini column and repeat the step 5 for centrifuge. Discard the filtrated fluid.
8. Now add 500µl of Tube B to mini column. Repeat the step 5 for centrifugation and discard the filtrated fluid.
9. Add 500µl of Tube C to mini column. Repeat centrifugation for 3 min at13000rpm and discard the filtrated fluid.
10. add 200µl of tube c to mini column. Repeat the step 5 for centrifugation and discard the filtrated fluid.
11. Centrifuge the mini column for 1 min at13000rpm to dry the matrix. Discard the used collection tube.
12. Now put the mini column (filter part) in a new 1.5 ml collection tube.
13. Add 50µl - 100µl of Tube E **(pre-warmed to 70°C)** to the mini column.
14. Now keep this at room temperature for two minutes.
15. Centrifuge this at 13000rpm for one minute.
16. Now you have fluid in the collection tube. This is your isolated DNA. This can be used to conduct different assays. Store your DNA at -20°C for long term application.

How to do the isolation from human blood samples (This protocol can be used for plasma, serum, cell cultures, vaccines and any body fluid):

1. Add 300µl of Tube A, 150µl of human blood /plasma/serum and 20µl of Tube-K in one tube. (Hint: Blood samples will create red colour because of lysis of RBC; volume of sample can be increased to 250 ul for plasma / serum to increase the amount of isolated DNA. Isolation can be done from animal samples)
2. Incubate at 56°C for 20-30 minutes. Add to this 400µl of tube G. Incubate at 70°C for 5 minutes.
3. Add to this 400µl of molecular ethanol and do the vortexing.
4. Take a mini column in one collection tube and add 600µl of above made solution to this mini column.
5. Centrifuge this for one minute at 11000rpm. Discard the filtrated fluid.
6. Add the rest of your remaining fluid in this mini column and repeat the step 5 for centrifuge. Discard the filtrated fluid.
7. Now add 500µl of Tube B to mini column. Repeat the step 5 for centrifugation and discard the filtrated fluid.
8. Add 500µl of Tube C to mini column. Repeat the step 5 for centrifugation and discard the filtrated fluid.
9. Add 200µl of tube C to mini column. Repeat centrifugation for 3 min at 13000rpm and discard the filtrated fluid.
10. Centrifuge the mini column for 1 min at 13000rpm to dry the matrix. Discard the used collection tube.
11. Now put the mini column (filter part) in a new 1.5 ml collection tube.
12. Add 50µl - 100µl of Tube E **(pre-warmed to 70°C)** to the mini column.

13. Now keep this at room temperature for two minutes.
14. Centrifuge this at 13000rpm for one minute.
15. Now you have fluid in the collection tube. This is your isolated DNA. This can be used to conduct different assays. Store your DNA at -20°C for long term application.

How to do the isolation from tissue:

1. Add 300µl of Tube A and 15 - 20µl of Tube K to 1-3 small pieces of the tissue in one tube. (Hint: Mouse tail or mouse ear samples can be processed)
2. Incubate at 56°C for 20-30 minutes. Add to this 400µl of tube G. Incubate at 70°C for 5 minutes.
3. Add to this 400µl of molecular ethanol and do the vortexing.
4. Take a mini column in one collection tube and add 600µl of above made solution to this mini column.
5. Centrifuge this for one minute at 11000 rpm. Discard the filtrated fluid.
6. Add the rest of your remaining fluid in this mini column and repeat the step 5 for centrifuge. Discard the filtrated fluid.
7. Now add 500µl of Tube B to mini column. Repeat the step 5 for centrifugation and discard the filtrated fluid.
8. Add 500µl of Tube C to mini column. Repeat the step 5 for centrifugation and discard the filtrated fluid.
9. add 200µl of tube C to mini column. Repeat centrifugation for 3 min 13000rpm and discard the filtrated fluid.
10. Centrifuge the mini column for 1 min 13000rpm to dry the matrix. Discard the used collection tube.
11. Now put the mini column (filter part) in a new 1.5 ml collection tube.
12. Add 50µl - 100µl of Tube E **(pre-warmed to 70°C)** to the mini column.
13. Now keep this at room temperature for two minutes.
14. Centrifuge this at 13000rpm for one minute.
15. Now you have fluid in the collection tube. This is your isolated DNA. This can be used to conduct different assays. Store your DNA at -20°C for long term application.

How to do the isolation from bacterial colonies:

1. Add 300µl of Tube A and 15 - 20µl of Tube K to containing bacterial colonies with loop or wooden stick in one tube. (Hint: Bacterial colonies can be diluted in 50-150 µl water or PBS in a tube also.)
2. Incubate at 56°C for 20-30 minutes. Add to this 400µl of tube G. Incubate at 70°C for 5 minutes.
3. Add to this 400µl of molecular ethanol and do the vortexing.
4. Take a mini column in one collection tube and add 600µl of above made solution to this mini column.
5. Centrifuge this for one minute at 11000 rpm. Discard the filtrated fluid.
6. Add the rest of your remaining fluid in this mini column and repeat the step 5 for centrifuge. Discard the filtrated fluid.
7. Now add 500µl of Tube B to mini column. Repeat the step 5 for centrifugation and discard the filtrated fluid.
8. Add 500µl of Tube C to mini column. Repeat the step 5 for centrifugation and discard the filtrated fluid.
9. add 200µl of tube C to mini column. Repeat centrifugation for 3 min 13000rpm and discard the filtrated fluid.

10. Centrifuge the mini column for 1 min 13000rpm to dry the matrix. Discard the used collection tube.
11. Now put the mini column (filter part) in a new 1.5 ml collection tube.
12. Add 50µl - 100µl of Tube E **(pre-warmed to 70°C)** to the mini column.
13. Now keep this at room temperature for two minutes.
14. Centrifuge this at 13000rpm for one minute.
15. Now you have fluid in the collection tube. This is your isolated DNA. This can be used to conduct different assays. Store your DNA at -20°C for long term application.

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

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| **Suggestion:**<br>**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.**<br><br>**Last update: 25-08-2018**<br>**v1.2** | **Genekam Biotechnology AG**<br>**Duissernstr.65a**<br>**47058 Duisburg**<br>**Germany**<br>**Tel. (+49) 203 / 555858-31,-32,-33**<br>**Fax (+49) 203 / 35 82 99**<br>**anfrage@genekam.de**<br>**http://www.genekam.de** |