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MADE IN GERMANY

Lot.

MANUAL

Ref. SB0001 – SB0004

Expiry date: 1 year
Store at 4^0C

GENEKAM UNIVERSAL DNA ISOLATION KIT

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

Contents:

- Tube A (lysis buffer)
- Tube K (proteinase K)
- Tube B (washing buffer 1)
- Tube C (washing buffer 2)
- Tube E (Elutionsbuffer)
- 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 30µl of Tube K to the sample in the tube.
2. Incubate this at 56 ^{O}C for 3 hours or overnight depending on your sample.
3. Add 300µl of Tube A and do vortexing. Incubate at 70 ^{O}C for 10 minutes. Add 300µl of molecular ethanol and do the vortexing.
4. Take a mini column in one collection tube (2 ml) and add 600µl of above made solution to this minicolumn.
5. Centrifuge this for one minute at 11000 g. 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 400µl of Tube B to minicolumn. Repeat the step 5 and discard the filtrated fluid.
8. Add 500µl of Tube C to minicolumn. Repeat the step 5 for centrifugation and discard the filtrated fluid.
9. Centrifuge the minicolumn to do the dry centrifuge. Discard the used collection tube.
10. Now put the mini column (filter part) in a new 1.5 ml collection tube.
11. Add 100µl of Tube E **(Prewarmed to 70°C)** to the minicolumn.
12. Now keep this at room temperature for one minute.
13. Centrifuge this at 11000 for one minute.
14. 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.

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2. Add 400µl of PBS (this is buffer) and 30µl of Tube K to the sample in the tube.
3. Incubate this at 56 ^{O}C for 3 hours.
4. Add 400µl of Tube A and do vortexing. Incubate this at 70 ^{O}C. Centrifuge at 11000 g for 1 minute and remove the head of buccal swab carefully. Now you have fluid in the tube. Add to this fluid 400µl of molecular ethanol.
5. Take a mini column in one collection tube (2ml) and add 600µl of above made solution to this minicolumn.
6. Centrifuge this for one minute at 11000 g. 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 400µl of Tube B to minicolumn. Repeat the step 5 and discard the filtrated fluid.
9. Add 500µl of Tube C to minicolumn. Repeat the step 5 for centrifugation and discard the filtrated fluid.
10. Centrifuge the minicolumn to do the dry centrifuge. Discard the used collection tube.
11. Now put the mini column (filter part) in a new 1.5 ml collection tube.
12. Add 100µl of Tube E **(Prewarmed to 70°C)** to the minicolumn.
13. Now keep this at room temperature for one minute.
14. Centrifuge this at 11000 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 human blood samples:

1. Add 300µl of Tube A , 150µl of human blood and 30µl of Proteinase-K in one tube.
2. Incubate at 70 ^{O}C for 10 minutes.
3. Add to this 300µl 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 minicolumn.
5. Centrifuge this for one minute at 11000 g. 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 400µl of Tube B to minicolumn. Repeat the step 5 and discard the filtrated fluid.
8. Add 500µl of Tube C to minicolumn. Repeat the step 5 for centrifugation and discard the filtrated fluid.
9. Centrifuge the minicolumn to do the dry centrifuge. Discard the used collection tube.
10. Now put the mini column (filter part) in a new 1.5 ml collection tube.
11. Add 100µl of Tube E **(Prewarmed to 70°C)** to the minicolum.
12. Now keep this at room temperature for one minute.
13. Centrifuge this at 11000 for one minute.
14. 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 bird blood:

1. 1 Add 300µl of Tube A, 20µl of bird blood and 30µl of Proteinase-K in one tube.
2. Incubate at 70^{O}C for 10 minutes.
3. Add to this 300µl of molecular ethanol and do the vortexing.
4. Take a mini column in one collection tube (2 ml) and add 600µl of above made solution to this minicolumn.
5. Centrifuge this for one minute at 11000 g. 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 400µl of Tube B to minicolumn. Repeat the step 5 and discard the filtrated fluid.
8. Add 500µl of Tube C to minicolumn. Repeat the step 5 for centrifugation and discard the filtrated fluid.
9. Centrifuge the minicolumn to do the dry centrifuge. Discard the used collection tube.
10. Now put the mini column (filter part) in a new 1.5 ml collection tube.
11. Add 100µl of Tube E **(Prewarmed to 70°C)** to the minicolum.
12. Now keep this at room temperature for one minute.
13. Centrifuge this at 11000 for one minute.
14. 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 30µl of Proteinase-K to one small piece of the tissue in one tube.
2. Incubate at 56 ^{O}C for 1 hours or overnight. Do the vortexing. Incubate at 70 degree for 10 minutes.
3. Add to this 300µl of molecular ethanol and do the vortexing.
4. Take a mini column in one collection tube (2ml) and add 600µl of above made solution to this minicolumn.
5. Centrifuge this for one minute at 11000 g. 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 400µl of Tube B to minicolumn. Repeat the step 5 and discard the filtrated fluid.
8. Add 500µl of Tube C to minicolumn. Repeat the step 5 for centrifugation and discard the filtrated fluid.
9. Centrifuge the minicolumn to do the dry centrifuge. Discard the used collection tube.
10. Now put the mini column (filter part) in a new 1.5 ml collection tube.
11. Add 100µl of Tube E **(Prewarmed to 70°C)** to the minicolumn.
12. Now keep this at room temperature for one minute.
13. Centrifuge this at 11000 for one minute.
14. 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.

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: 14-06-2012** **v1.1**	**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**