

Lot.

Ref. SB0079

MANUAL

Expiry date: 1 year

Store at 4°C

GENEKAM RNA ISOLATION KIT (VIRUS)

-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 2µl of Tube K to the sample in the tube.
2. Incubate at 56°C for 20-30 minutes. Add to this 200µl of tube A. Incubate at 70°C for 5 minutes.
3. Add to this 500µl 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 for centrifugation 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. add 200µl 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.

Tips:How to do the isolation from the buccal swabs:

1. Cut the head of buccal swabs.
2. Incubate at 56°C for 20-30 minutes. Add to this 200µl of tube A. Incubate at 70°C for 5 minutes.
3. Add to this 500µl 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 for centrifugation 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. add 200µl 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 (This protocol can be used for plasma, serum, all cell cultures, vaccines and any body fluid):

1. Add 300µl of Tube A , 150µl of human blood and 20µl of Tube-K in one tube.
2. Incubate at 56°C for 20-30 minutes. Add to this 200µl of tube A. Incubate at 70°C for 5 minutes.
3. Add to this 500µl 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 for centrifugation 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. add 200µl 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 tissue:

1. Add 300µl of Tube A and 20µl of Tube K to one small piece of the tissue in one tube.
2. Incubate at 56°C for 20-30 minutes. Add to this 200µl of tube A. Incubate at 70°C for 5 minutes.
3. Add to this 500µl 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 for centrifugation 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. add 200µl 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 minicolum.
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.

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.

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