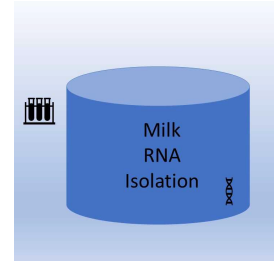


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RNA-Isolation from milk samples for virus analysis

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Protocol status: Working

We use this protocol and it's working

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Disclaimer

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Abstract

This protocol is about a method to isolate RNA from the milk samples to conduct the viral analysis, for example avian Influenza Virus H5N1, Measles, Zika Virus, Dengue Fever Virus etc. in human and other mammalian species. The isolated RNA can be used for conducting the molecular biological analysis like conventional as well as real time PCR, nucleic acid based nanoparticle assays etc.

Image Attribution

Milk RNA Isolation

Guidelines

Read the manual before use.
Check the contents of the kit.

Precautions:

- The kit is only for in vitro diagnostic use.
- The kit may only be used by trained persons.
- The user must read the manual carefully.
- The kit should not be used after expiry date.
- The user should work very cleanly when isolating.
- Decontaminate the instruments regularly (once a week).
- The user should wear protective gloves and laboratory clothing.



Materials

-SB0079 - Genekam Universal RNA-Isolation Kit (INT) for human samples;

-UDI-DI: 0426242043077 - CE

for other mammalian samples: **MB-SB0079 RNA isolation kit (Virus)** from Microboss Hightech GmbH

Materials required:

-Molecular Ethanol

-Pipettor and Pipette tips

-Heat block

-Centrifuge

-Vortexer

Safety warnings

- ! -Keep the kit away from sun light.
- If the package and the bottles are damaged don't use the kit.
- Read the material safety data sheet.

Before start

Read the manual and note the important points e.g. temperature and centrifugation speed.



- 1 Adjust the temperature of heat block.
- 2 Add 300µl of Tube A (Lysis buffer I) and 15µl of Tube K (Proteinase K) to the 100µl milk sample in the tube.
- 3 Incubate at 56°C for 20-30 minutes. Add to this 400µl of Tube G (Lysis buffer II).
Incubate at 70°C for 5 minutes. 25m
- 4 Add to this sample 400µl of molecular ethanol and shake with the vortexer.
- 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. 1m
- 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 (Washing buffer I) to mini column. Repeat the step 5 for centrifugation and discard the filtrated fluid. 1m
- 9 Add 500µl of Tube C (Washing buffer II) to mini column. Repeat the step 5 for centrifugation and discard the filtrated fluid. 1m
- 10 Add 200µl of Tube C to mini column. Repeat centrifugation for 3 min at 13000RPM and discard the filtrated fluid. 3m
- 11 Centrifuge the mini column for 1 min at 13000RPM to dry the matrix. Discard the used collection tube. 1m
- 12 Now put the mini column (filter part) in a new 1.5 ml collection tube.
- 13 Add 100µl of Tube E (**pre-warmed to 70°C**) to the mini column.



14 Now keep this at room temperature for two minutes.

2m

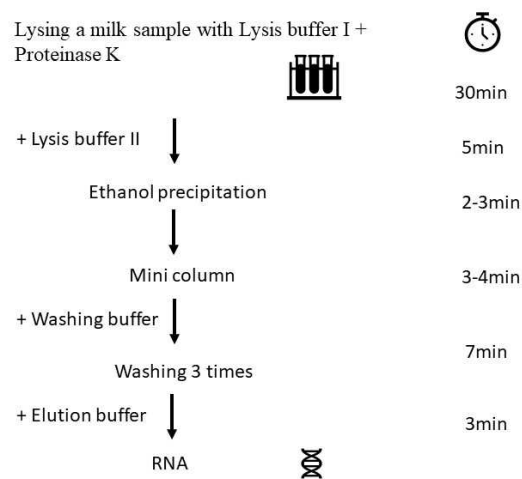
15 Centrifuge this at 13000RPM for one minute.

1m

16 Now the user has fluid in the collection tube. This is isolated RNA. This can be used to perform different assays. The RNA should be stored at -20°C for long-term use.

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Picture 1: Scheme of milk RNA isolation method of around 50-55 minutes.



Scheme of the milk isolation method of around 50-55 minutes