Ref. MB-SB0101

Expiry date: 1 year
Store at 4^0C

RNA ISOLATION KIT (MAGNETIC BEADS)

-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 M (Magnetic beads)
- Tube E (Elution buffer)

Chemicals and equipments needed:

- Pipettes and Pipette tips
- Heat block
- Centrifuge (for some cases)
- Magnetic rack (Genekam or other manufacturer)
- RNase free tube
- Microtubes (1.5 or 2ml)

Procedure:

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

1. Add 100-250µl plasma / serum / cell culture media containing viruses or transfected cells / vaccine / body fluid and 600µl of tube A together in one tube. Add 15µl of tube M (magnetic beads) to it.

2. To it add 20µl of tube K.

3. Incubate at room temperature for 8 minutes.

4. Separate the beads with Genekam magnetic rack keeping it for 2 minutes. Discard the supernatant while gently dropping the fluid in waste collecting container if you are using Genekam Rack, where the tube fits very tightly, user can drop the supernatant without any pipettor as this makes the application of magnetic very simple and easy.

5. Hint: if user is using other than Genekam magnetic rack, user may need to use the pipettor to remove the fluid!

6. Add 500µl of tube B (add 10µl of tube K; it should be added before the use as it should be freshly preapared) and suspend the beads. Keep at room temperature for 10 minutes. Separate beads with magnet rack. Discard the supernatant while gently dropping the fluid in a waste container.

7. Add 500µl of tube B (add 10µl of Tube K to it. It should be freshly prepared before the use) and suspend the beads in it. Keep at room temperature for 1 minute. Separate the beads with magnet and discard the supernatant while gently dropping the fluid in a container. There may be some fluid left at the bottom of tubes, please remove this fluid with pipettor before adding elution buffer.

8. Add 100µl of tube E and suspend the beads in this elution solution. Incubate it at 80° C for 10 minutes (during heating, remix it or shake it). Separate the beads with magnet rack while collecting the elute with a pipettor und store the supernatant containing RNA in RNAase free tube while discarding the beads.

Hint: 1. How to isolate RNA from blood samples: to 50µl of blood, add 760µl of tube A and 15µl of tube M (magnetic beads). To these, add 20µl of tube K. Now keep it at room temperature for 10 minutes and proceed ahead with number 4 of standard step onwards.

2. How to isolate RNA from buccal swabs or nasal swab: add 300 µl of tube A to buccal swab. Add to it 25µl of tube K. Keep it at 56 ^{0}C for 10 minutes. Remove the buccal swabs (very important!) and add 300µl of tube A to it. Now proceed with step 2 of standard step (i.e. adding 15 to 20 µl of beads) till elution. Similarly one can isolate the RNA from a piece of tissue. If the tissue piece is not dissolved, collect the supernatant in another tube to proceed with the step 8.

3. How to isolate RNA from cell pellet e.g. mononuclear cells or other cells or from vaccines (50µl depending on the number or concentration of cells, there is no need to count the number of cells): add cell pellet to 600µl of tube A in a tube. To this, add 15µl of tube M. To them add 20µl of tube K. Now proceed with step 3 of standard step. Similarly bacterial colonies (pick the colonies with loop and add to 600µl of Tube A or 20ul of bacterial colonies as cell suspension) can be used to isolate the RNA.

4. How to isolate the RNA from tissue: add 2-3 tissues pieces e.g. umbilical cord wall or placenta. Add to it, 600µl of Tube A for 8 Minutes along with 15µl Proteinase K. Remove the supernatant (centrifuge it, if needed!) and put it in another tube. Now add 20µl magnetic beads to this. Proceed with step 3 (i.e. Keep it for 8 minutes at room temperature) of standard step onwards.

These are only a few examples. User can try the kit with many different kinds of samples also!

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: 17-04-2020** **v1.5**	**Genekam Biotechnology AG** **Duissernstr. 65a** **47058 Duisburg** **Germany** **Tel. (+49) 203 / 555858-31,-32,-33** **Fax (+49) 203 / 35 82 99** **www.genekam.de**