User-Developed Protocol:

Isolation of RNA from leukocytes in milk using QIAGEN® RNeasy® Kits

This procedure has been adapted by customers and is for isolation of RNA from leukocytes in milk using the RNeasy® Mini Kit or RNeasy 96 Kit with QIAshredder™ homogenizers. It has been used by customers for the isolation of bovine viral diarrhoea virus (BVDV) RNA from cow milk samples. **It has not been thoroughly tested and optimized by QIAGEN.**

One milliliter of milk contains approximately 50,000–200,000 leukocytes (with an average of approximately 100,000 leukocytes). In a BVDV-infected animal up to 1–30% of the leukocytes may be infected with the virus. Each infected leukocyte typically contains 10–10,000 BVDV entities.

Please be sure to read either the *RNeasy Mini Handbook* or the *RNeasy 96 Handbook* carefully before beginning this procedure.

Important notes before starting

- Use raw milk (not UHT or pasteurized milk) for the isolation procedure.
- All centrifugation steps are carried out at room temperature (15–25°C).
- QIAGEN® Centrifuges 4-15C and 4K15C can be used for both sample tubes and Flat-Bottom Blocks*.

Protocol 1. Isolation of RNA from leukocytes in milk using the RNeasy Mini Kit

1. **Milk should be stored at 2–8°C prior to leukocyte preparation. After overnight storage, a fatty layer of cream will form. This should be removed from the top of the milk using a water suction pump and discarded.**
2. **Homogenize the milk by heating at 37°C for 15 min and mix.**
3. **Transfer 15 ml milk into a sample tube (e.g., a BD Falcon™ tube).**

   Alternatively: Up to 50 milk samples can be pooled and tested. When pooling samples, the maximum volume of each sample is 1 ml. Transfer the entire pooled volume (i.e., up to 50 ml) into a sample tube and proceed with step 4.
4. **Centrifuge sample tubes at approximately 6000 x *g* for 10 min to sediment the leukocytes.**
5. **A solid fatty clot will be present in the centrifuged samples; this should be punctured and discarded together with the milk supernatant.**

** The Plate Rotor 2 x 96 (cat. no. 81031) is required for accommodating 96-well blocks and plates on QIAGEN Centrifuges 4-15C and 4K15C. Other rotors, available from Sigma Laborzentrifugen GmbH, are necessary for accommodating tubes on these centrifuges.*

6. **Add 7.5 ml PBS to the sample tubes. Mix, and centrifuge at 6000 x *g* for 10 min to wash the leukocyte pellet. Discard the resulting supernatant.**

7. **Add 1 ml PBS to the leukocyte pellets. Mix, and centrifuge at 6000 x *g* for 10 min.**

8. **Resuspend leukocyte pellets in 350–600 µl Buffer RLT and mix by vortexing.**
   Note: After this step, lysates can be stored at –70°C.

9. **Load each lysate onto a QIAshredder Spin Column (provided in the RNeasy Plant Mini Kit, and also available separately from QIAGEN, cat. no. 79654 or 79565) placed in a 2 ml collection tube. Centrifuge the QIAshredder columns at maximum speed for 2 min.**

10. **Carefully transfer the supernatant of the flow-through fraction to a new microcentrifuge tube without disturbing the cell-debris pellet. Use only this supernatant in subsequent steps.**

11. **Add 1 volume of 70% ethanol to the cleared lysate. Mix by pipetting, and apply the sample onto an RNeasy Mini Spin Column placed in a 2 ml collection tube.**
    It may be necessary to load the samples in two aliquots.

12. **Continue with the RNeasy Mini Protocol for Isolation of Total RNA from Animal Cells in the *RNeasy Mini Handbook*, from step 5.**

Protocol 2. Isolation of RNA from leukocytes in milk using the RNeasy 96 Kit

1. **Milk should be stored at 2–8°C prior to leukocyte preparation. After overnight storage, a fatty layer of cream will form. This should be removed from the top of the milk using a water suction pump and discarded.**
2. **Homogenize the milk by heating at 37°C for 15 min and mix.**
3. **Pipet 1.8 ml of each milk sample into the wells of a Flat-Bottom Block.**
4. **Seal the block with tape (provided) and centrifuge at approximately 6000 x *g* for 10 min to sediment the leukocytes.**
5. **Remove the tape. A solid fatty clot will be present in the centrifuged samples; this should be punctured and discarded together with the milk supernatant.**

   Residual liquid in Flat-Bottom Blocks can be removed by tapping the blocks firmly on absorbent paper towels.
6. **Add 1 ml PBS to each well of the Flat-Bottom Block. Seal the block with tape and shake at 800 rpm for 5 min to resuspend the leukocyte pellets. Centrifuge the block at 6000 x *g* for 10 min.**
7. **Remove the tape. Add 350–600 µl Buffer RLT to each well of the block. Seal the block with tape and shake at 1000 rpm for 15 min to resuspend the leukocytes.**

   Note: After this step, lysates can be stored at –70°C.
8. **Load each lysate onto a QIAshredder Spin Column placed in a 2 ml collection tube. Centrifuge the QIAshredder columns at maximum speed for 2 min.**
9. **Carefully transfer the supernatant of the flow-through fraction to a new microcentrifuge tube without disturbing the cell-debris pellet. Use only this supernatant in subsequent steps.**
10. **Add 1 volume of 70% ethanol to each cleared lysate. Mix by pipetting, and apply the samples into the wells of an RNeasy 96 Plate.**

    It may be necessary to load the samples in two aliquots.
11. **Continue with one of the RNeasy 96 Protocols for Isolation of Total RNA from Animal Cells in the *RNeasy 96 Handbook*, from step 5.**

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