



## Systems Virology of Emerging Respiratory Viruses

### TM001.0P - RNA Extraction from Infected Mouse Tissue

For tissues to be used for genomic analysis, it is important to adhere to a standardized protocol for harvest. Time is limited because RNA begins to degrade immediately post-ethanization (anesthesia will not adversely affect tissues for RNA isolation). Whole tissues collected from biopsy or at necropsy must be preserved quickly to prevent as much RNA degradation as possible.

To ensure this one should harvest tissue from 1 animal at a time, have tubes and vessels all labeled and filled with appropriate solutions before starting and do all of the following steps as quickly as is possible.

1. Harvest tissue from animal. Use lobe 4 & 6 for RNA (see diagram below)
2. Briefly rinse tissue in a reservoir of cold (4°C) saline or PBS
3. Follow the RNAlater protocol described below

#### RNAlater Protocol

1. Cut tissue into small chunks (<0.5cm in any single dimension)
2. Place immediately into a 10-20 volumes (w/v) (e.g. 100mg/ml) RNAlater (Ambion, Cat # AM7021).
2. Samples MUST be completely submerged in a 10x volume of RNAlater to prevent the degradation of RNA. RNAlater does not expand upon freezing, so fill the vial to the very top. Invert the vial to ensure all tissue is freely moving but immersed. RNAlater must completely permeate the samples to be effective.
3. Incubate the samples thoroughly immersed in RNAlater solution overnight at 4°C.
4. After the 4°C incubation, samples should be stored at -20°C or -80°C for archiving or until ready to be homogenized.

**Note: These samples are still potentially infectious**

#### Homogenization Protocol- perform in biosafety hood-

(This may vary to some degree depending on method of homogenization.)

For thorough, fast homogenization, it is preferable to use a rotor-stator homogenizer such as the Omni Homogenizer (has disposable tips), Polytron, etc. If the Omni homogenizer is used, there is no need to wash between samples and one can purchase pre-drilled caps and tubes to minimize aerosols. If using a Polytron type homogenizer, Clean the homogenizer with RNase Zap (Ambion, Cat # AM9780), then wash with dH<sub>2</sub>O, 95% Ethanol and finally with 1 ml of Trizol reagent or Buffer RLT (every time run Polytron at full speed for about a minute) between every sample. Other means of homogenization are

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acceptable if they can be performed quickly and the tissue can be thoroughly homogenized.

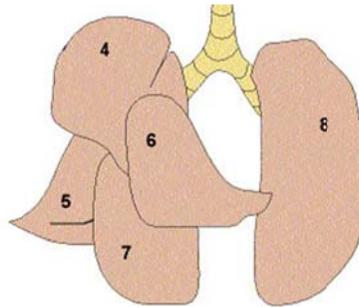
1. Remove sample from RNAlater and dab it on a kimwipe to remove excess liquid.
  2. Wash with a small volume of Trizol.
  3. Quickly weigh the tissue.
  4. Transfer to a 15 ml Falcon tube with 10-20 volumes (w/v) Trizol.
  5. Homogenize the sample several times at maximum speed (start at low speed and gradually increase), each time for about 30 sec (to prevent overheating). Continue homogenizing until tissue is completely disrupted.
  6. Place tube on ice (4°C) until all samples have been processed.
  7. Transfer to a labeled 1.5 or 2ml tube (preferably with O-ring screw cap lid)
- Note: now the material should no longer be infectious and can be taken out of BSL2/3**
8. Freeze samples on dry ice and store at -80°C until shipping.
  9. Ship sample on dry ice with appropriate safety documentation.

Lobe 4/6 – RNA

Lobe 5 – Protein

Lobe 7 – titer

Lobe 8 - pathology



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