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MHV Tissue Titering Protocol V.1

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Abstract

This protocol is for isolating total nucleic acid from soft tissues in mice, for subsequent analysis of Viral RNA levels

Protocol materials

- RPMI 0% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**R7755
- 2.0 mm Zirconia beads **BioSpec Products Catalog #**11079124ZX
- 1.1 mL Polypropylene Cluster Tubes, 12-Tube Strip Format, Nonsterile **VWR International (Avantor) Catalog #**89005-574
- 12-Well Cluster Tube Caps **VWR International (Avantor) Catalog #**89005-728
- Sterile 24 Well Plate **VWR International (Avantor) Catalog #**103348-844
- DTT **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D0632
- Buffer RLT Plus **Qiagen Catalog #**1053393
- Deep 96 Well Plate **VWR International (Avantor) Catalog #**10011-940
- EconoSpin 96 Well DNA & RNA Binding Plate **Epoch Life Science Catalog #**2020-001
- Ethyl alcohol, Pure **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E7023
- Buffer RW1 **Qiagen Catalog #**1053394

Troubleshooting

Day -1 (Or earlier): Design Well Layout for Tissue Collection tubes

1 Design the 96-well plate layout in which you will process (and eventually store) your samples 15m

Day -1: Prepare tissue collection tubes and plate

2 Label a sufficient number of 1.1 mL 12-well cluster tubes, and then place them in a new rack. 10m

1.1 mL Polypropylene Cluster Tubes, 12-Tube Strip Format, Nonsterile **VWR International (Avantor) Catalog #**89005-574

3 Retrieve enough 12-well cluster tube caps for your cluster tubes

12-Well Cluster Tube Caps **VWR International (Avantor) Catalog #**89005-728

4 Add 5-10 2.0 mm Zirconia beads to each tube that will have tissue in it 5m

2.0 mm Zirconia beads **BioSpec Products Catalog #**11079124ZX

5 Add 650 µL RPMI 0% to each bead containing tube 5m

RPMI 0% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**R7755

6 Cover rack and tubes with resealable plate mat and leave in 4 °C until ready to use 5m

7 Add 6 mL (Approx) of RPMI 0% to a 24 well deep well plate

RPMI 0% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**R7755

Sterile 24 Well Plate **VWR International (Avantor) Catalog #**103348-844

Day 0 1h 30m

8 Dissect the mouse and open the abdominal cavity without disturbing the adipose tissue

9 Collect mesenteric lymph nodes, Peyer's patches, and proximal colon

9.1 Orient mesenteric adipose tissue so mesenteric lymph nodes (mLN) are easily identifiable and place all mesenteric lymph nodes (with capsule) in the 1.1 cluster tube

9.2 Extract all the Peyer's patches and place them in the cluster tube

9.3 Cut 0.5 cm of proximal colon (the part of the colon that connects to the cecum)

10 Seal tube tightly with cap. Put whole weight on it, if necessary; it must be sealed by any means necessary 1m

11 Bead beat plate in SPEX MiniG Tissue Homogenizer for 5 min at 1500 rpm 5m

1500 rpm, Room temperature , 00:05:00

Equipment

new equipment	NAME
SPEX	BRAND
SP 1600	SKU
MiniG 1600 Automated Tissue Homogenizer and Cell Lyser	SPECIFICATIONS

11.1 Seal the bead beater with the 96 well plate mat and a paper towel to examine if there is serious leakage

12 Thaw [M] 2 Molarity (M) DTT to make a sufficient amount of RLT + 50 mM DTT

	A	B	C
	Number of Samples	Amount of RLT Plus (mL)	Amount of 2 M DTT (uL)
	30	12.5	250

Spreadsheet to calculate how much RLT Plus and DTT will be needed

DTT **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D0632

Buffer RLT Plus **Qiagen Catalog #**1053393

13 Aliquot 350 uL of RLT+DTT buffer to appropriate wells in a new 96 well deep well plate

Deep 96 Well Plate **VWR International (Avantor) Catalog #**10011-940

14 Once the plate is done beating: 3000 x g, Room temperature, 00:02:00

2m

15 While the plate is spinning, set up the Multi-well Plate Manifold with a 96-well silica plate on top

EconoSpin 96 Well DNA & RNA Binding Plate **Epoch Life Science Catalog #**2020-001

Equipment

Multi-well plate manifold	NAME
Vacuum Manifold	TYPE
Pall	BRAND
5017	SKU
https://www.cytivalifesciences.com/en/us/shop/lab-filtration/manifolds-and-accessories/microbiology-manifold/vacuum-manifold-and-accessories-p-36407	LINK

16 Once the plate is done spinning, add 100 uL of tissue supernatant to theRLT-DTT plate

16.1 Use the pipet that you transfer the organ homogenate to pipet up and down to mix the RLT with the homogenate

16.2 Once the 100 uL has been transferred to the RLT-DTT plate, the RNA is stable, and you can transfer the 200 uL of the remaining homogenate to another deep 96-well plate for FFU titers Deep 96 Well Plate **VWR International (Avantor) Catalog #**10011-940

17 Add 350 uL of pure ethanol to the RLT-DTT well plate and do not mix here
Ethyl alcohol, Pure **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E7023

18 Turn the vacuum on to prepare the silica plate

19 With a new pipet tip, mix the RLT+Ethanol solution and transfer to the silica plate

19.1 Transfer the solution with the same pipet you mix the ethanol with the pipet

19.2 The solution should take ~1 minute, usually less to suck through

20 Wash the silica plate with 350 uL RW1 Buffer
Buffer RW1 **Qiagen Catalog #**1053394

21 Wash the silica plate with 800 uL RPE Buffer (10 mM Tris-Cl + 80% Ethanol)

22 Dry the plate 3000 x g, Room temperature, 00:02:00 2m

23 Place plate onto an elution skirted plate.

23.1 Elute by adding 75 uL of DEPC treated water to the wells and
3000 x g, Room temperature, 00:02:00

2m