**Objective:** Recipe for preparing large batches of master mix for one-step qRT-PCR analysis of West Nile mosquito and raptor samples.

**Notes:**

* Adapted from Michael Young’s WNV-s Master Mix recipe.
* See Reagents & Supplies excel sheet for catalogue numbers and Spec sheets for primer/probe sequences.
* Primer/Probe stocks will be 100 uM, make a 1:10 dilution of each stock in nuclease-free H2O for 10 uM reagents. Volume of dilution will vary depending on the batch size.
* Premade master mix aliquots may be made in any size volume, provided the total quantity of reagent/well remains consistent and the master mix used across one plate is identical. The volume of reagents in the master mix for one reaction/well is as follows:

|  |  |
| --- | --- |
| **Reagent** | **uL per well/reaction** |
| Express qPCR Supermix, Universal | 10 |
| Nuclease-free H2O | 1.5 |
| 10uM Forward Primer | 1 |
| 10uM Reverse Primer | 1 |
| 10uM FAM Probe | 0.5 |
|  |  |
| Superscript Mix\* | 1 |
| Unknown RNA\*\* | 5 |

\*This master mix does not contain the Express SuperScript Mix for One-step qPCR, this reagent must be added immediately prior to use of the master mix (27 uL into 27x tubes, 13 uL into 13x, etc.)

\*\*Unknown Sample RNA added to each well after loading master mix.

**Biosafety precautions:**

* Smaller batches may be made at bench, larger batches made in BSC.

**Materials:**

* Express qPCR Supermix, Universal, 5mL bottle
* Nuclease-free H2O
* 10 uM Forward primer
* 10 uM Reverse primer
* 10 uM FAM probe

**Protocol:**

1. Vortex Supermix and add 4,819.5 uL to a 50 mL conical tube.
2. Add 722.9 uL nuclease-free H2O to the tube.
3. Vortex and spin down the Forward/Reverse primers and add 482 uL of each to the tube.
4. Vortex and spin down the FAM probe and add 241 uL to the tube.
5. Mix thoroughly.
6. Aliquot 378 uL into fifteen 1.7 mL tubes, label 27x.
7. Aliquot 182 uL into four 1.7 mL tubes, label 13x.
8. Store at -20º until needed.

**Results and analysis: N/A**