**RNA extraction KINGFISHER on brain tissue**

**\*\*Need to use Qiagen Tissue Shredder II to lyse the samples rapidly**

**Pre-steps**

* RNAzap your working area before stating
* Samples should always be on ice
* Ensure you wash solutions have the correct amount of Isopropanol or ethanol added. This will be done for every new Mirvana RNA kit. The wash solutions should be checked off.
* Turn on Kingfisher Flex, Scroll over to Red tab (I click to the right) and then scroll down to RNA, select the A27828\_FLEX\_Tissue\_Cells

**Prepare Reagents**

1) Prepare Turbo DNAse solution

Keep on ice until use

*For 1 sample*

48 uL magmax turbo DNA buffer

2 uL Turbo DNAse

*For 96 samples*

4.8 mL magmax turbo DNA buffer

200 uL Turbo DNAse

2) Prepare binding bead mix

Keep on ice until use

Vortex binding beads to start

For 1 sample

10 uL RNA binding beads

10 uL Lysis binding enhancer

For 96 samples

1 mL RNA binding beads

1 mL Lysis binding enhancer

**Preparing the lysis binding mix**

1) For every 100 uL of lysis buffer add .7 uL of 2-Mercaptoethanol (β-mercaptoethanol)

If you are using 50 mg of brain tissue, you need 1 mL of lysis buffer and 7 uL of 2-Mercaptoethanol

If you are doing 96 samples of 50 mg of tissue per sample you would need 100 mL of lysis buffer, and 7 mL of 2-Mercaptoethanol

**Lyse the tissue**

1) Take a maximum 50 mg of brain tissue and place it in a QIAGEN collection microtube ( Cat. No. 19560) .

For every 1 mg of tissue add 20 uL of lysis binding mix.

For 50 mg tissue you need 1 mL of lysis binding mix.

2) Add a stainless steel bead to the tube containing the tissue and the lysis binding mix.

3) Add a cap to the tube (collection microtube cap, Qiagen) and then remove the lid from the box

4) Encapsulate the box with the metal Qiagen tissue shredder adaptor

5) Place the box with samples with attached metel encapsulator in the Tissue Shredder II

6) Turn on the tissue shredder using the switch on the back

7) Use the following settings frequency (1/s) = 30, and Time = 1.00 min

8) Remove the sample place on Ice. You will only need some of the lysate you created for the next steps (100 uL). Put the rest of the lysate in the freezer. Conversely you can freeze all the sample and stop here

**Bind RNA**

1) If the samples are frozen at the previous step thaw them completely.

2) Vortex the lysate created in the previous section

3) Transfer 100 uL of the lysate to a 96 deep well plate, freeze the rest

4) Cover the plate and put on the shaker for 5 minutes, at max speed (10)

5) Add 100 uL of iso-proponal to each sample, cover the plate, shake for 2 min at speed 7

6) Add 20 uL of prepared binding bead mix, cover the sample, shake for 5 min at speed 7

**Make the plates**

1) Make the following plates while the other incubation steps are happening. Make them all in a deep well plate. Your kingfisher has the long deep well magnet so you need to do deep well plates. Label each well plate.

|  |  |  |  |
| --- | --- | --- | --- |
| **Plate ID** | **Plate position** | **Reagent** | **Volume per well** |
| Wash plate 1 | 2 | Wash Solution 1 | 150 uL |
| Wash plate 2 | 3 | Wash Solution 2 | 150 uL |
| DNAse plate | 4 | TURBO DNase Solution | 50 uL |
| Wash plate 3 | 5 | Wash Solution 2 | 150 uL |
| Wash plate 4 | 6 | Wash Solution 2 | 150 uL |
| Elution Plate | 7 | Elution Buffer | 50 uL |
| Tip comb | 8 |  |  |

Note: Your tip comb still needs to be inserted into a 96 well plate

2) Place all the plate on the machine in the order specified

**Run the samples**

1) Place your sample plate on the kingfisher, it goes in position 1, the kingfisher tells you were they go. Start the process

2) When prompted, about 30 minutes in, you remove the DNAse plate and add 50 uL of rebinding buffer and 100 uL of isoproponal. Do not pre-mix these, add them separately.

3) Put the plate back and re-start the process

4) When the process completes immediately remove the plate, nanodrop, and put in a storage plate that should go in the freezer (-80) for long term storage

Note: 100 uL of lysate yields approximately 50 ng/uL of RNA