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| **S.O.P. #** | 301.01 |
| **Title** | Copurification of DNA and RNA from tissue |
| **Effective Date** |  |

* **Purpose**

To describe the procedure to copurify DNA and RNA from frozen brain tissue

* **Equipment and Materials**
  + **Equipment**
    - Tissue Homogenizer (Omni International, #TH115)
    - Microcentrifuge (Fisher, #S39740)
    - P20 micropipette (Rainin, #PR-20)
    - P200 micropipette (Rainin, #PR-200)
    - P1000 micropipette (Rainin, #PR-1000)
  + **Materials**
    - AllPrep DNA/RNA kit (Qiagen, #80204)
    - Proteinase K (Qiagen, #19131)
    - RNase free DNase set (Qiagen, #79254)
    - 20 µl pipette tips (ASI, #FT1020)
    - 200 µl pipette tips (ASI, #FT1200)
    - 1000 µl pipette tips (ASI, #FT1250)
    - 2ml Eppendorf tubes (Eppendorf, #022600044)
    - 1.5ml Eppendorf tubes (Eppendorf, #022600028)
    - -mercaptoethanol (-ME; Millipore Sigma, #444203)
    - 200 proof Ethanol (Fisher, #BP2818)
* **Health and Safety**
  + - Refer to SOP 005.01 (proper laboratory attire)
* **Procedure**
  + Add 10µl of -ME to 1ml Buffer RLT plus before use. Can be stored at room temperature for up to 1 month
  + Add 4 volumes of 100% Ethanol to Buffer RPE for a working solution (if kit is new)

1. Harvest tissue into a 2ml Eppendorf. Do NOT use more than 30mg, but 5-10mg is preferred. Homogenize the tissue in 600µl Buffer RLT plus (with -ME). Lysate can be stored at -°C before further processing.
2. Centrifuge the lysate for 3min at max speed.
3. Carefully transfer the lysate to an **AllPrep DNA spin** **column** placed in a 2ml collection tube. Close lid gently, and spin for 1min at 8000 x g. If lysate does not pass through column, increase time and/or g.
4. Place the AllPrep DNA column in new 2ml collection tube. Store at 4°C for later DNA purification (during the DNase I treatment step). **SAVE THE FLOW THROUGH.**

**RNA purification**

1. Add 600µl of 70% ethanol to the flow through. Mix well by pipetting and immediately proceed to step 2.
2. Transfer 600µl of the sample, including any precipitate, to an **RNeasy spin column**. Spin for 1min at 8000 x g. If lysate does not completely pass through, increase time and/or g. Discard the flow-through. Repeat for remaining sample.
3. Add 350µl Buffer RW1 to RNeasy spin column. Spin for 1min at 8000 x g.
4. Perform DNase digestion (requires separate kit)
   1. For each column, mix 10µl DNase, 70µl Buffer RDD.
   2. Apply 80µl to the column.
   3. Incubate at room temperature for 15min with the lids **OPEN.**
   4. Add 350µl Buffer RW1. Spin for 1min at 8000 x g. Discard flow-through.
5. Add 500µl Buffer RPE. Spin for 1min at 8000 x g. Discard flow-through.
6. Add 500µl Buffer RPE. Spin for 2 min at 8000 x g. Discard flow-through.
7. To remove any remaining ethanol, place column in a new 2ml collection tube. Spin for 3 min at max speed. Discard flow-through.
8. Place column in new 1.5ml collection tube. Add 50µl RNase-free water directly to the spin column membrane (try to avoid the sides of the column). Incubate for 3 min at room temperature. Spin for 1min at 8000 x g.

**DNA purification** (perform during DNase digestion)

1. Add 500µl Buffer AW1 to the **AllPrep DNA spin column**. Spin for 1min at 8000 x g. Discard flow-through.
2. Add 500µl Buffer AW2. Spin for 2 min at max speed. Discard flow-through.
3. To remove any remaining ethanol, spin for 3 min at max speed. Discard flow-through.
4. Place column in new 1.5ml collection tube. Add 100µl Buffer EB directly to spin column membrane (try to avoid the sides of the column). Incubate for 3 min at room temperature. Spin for 1min at 8000 x g.