Rneasy mini protocol

I. Tissue disruption and homogenization
  A. Tissue removal from RNAlater
    1. For eye cups
      a. Remove eye cup from RNAlater with clean tweezers
      b. Dissect out retina
    2. For retina
      a. If in blue micropestle tube already, just pipette off RNAlater leaving tissue behind and add 600 ul buffer RLT
      b. If not, transfer retina to blue micropestle tube containing 600 ul buffer RLT with clean tweezers
      c. Should be less than 30 mg of tissue
        i. Weight of one retina from 5 mm eye is 20-30 mg
      d. Take to hood
        i. Add β-mercaptoethanol to RLT
          1. Add 6 ul for 0.6 ml RLT
  B. Tissue disruption
    1. Use clean blue pestle to disrupt tissue
      a. Do in HOOD
      b. If you have cellular debris (eye cup bits) spin suspension down
  C. Homogenization
    1. Pipette lysate onto QIAshredder spin column in 2 ml collection tube
      a. Spin 2 minutes at 12000g
        i. Max speed
        ii. Eppendorf 14000rpm = 16000g
      b. Remove column and cap tube
      c. Spin 3 minutes at max speed
II. Column purification
  A. Add 1 volume (600 ul) of 70% ethanol to clean tube (not supplied)
    1. Tranfer lysate and mix by pipetting
    2. Transfer to Rneasy minicolumn (700 ul at a time)
    3. Centrifuge 15s at ≥8000g
      a. 8000g Eppendorf = 9800 rpm
      b. Discard flow through
      c. Add rest and centrifuge
      d. Discard flow through
  B. Add 700 ul Buffer RW1
    1. Centrifuge 15s at ≥8000g
  C. Transfer to new 2 ml collection tube (supplied)
    1. Add 500 ul buffer RPE
      a. Need to add EtOH to buffer RPE before use
      b. Spin 15s at ≥8000g
      c. Discard flow through
    2. Add another 500 ul RPE
      a. Spin 1 min at ≥8000g

b. Discard flow through
c. Spin 2 min at 12000g

III. Elution

A. Transfer column to clean 1.5 ml tube (supplied)
1. Pipet 30-50 ul Rnase free water directly onto membrane
2. Spin 1 min at ≥8000g to elute
3. Repeat elution with second volume of 30-50 ul

IV. Things needed

A. Supplies to purchase
1. Blue micropestle tube and pestle
a. VWR KT749520-0090 $82 / 100
2. QIAshredder
a. Qiagen 79654 $57 / 50
3. RNEasy mini column kit
a. Qiagen 74104 $203 / 50
b. Qiagen 74106 $891 / 250

B. Reagents
1. Need β-ME to add to RLT
2. Add EtOH to RPE
3. Need 70% EtOH – RNAse free

Short protocol

Do spins in Eppendorf located in hood.

	Steps	Spins		
1.	Remove RNAlater			
2. Homog	Add retina to 0.6 ml RLT + 6ul βME Disrupt in blue tube with pestle			
	Add lysate to Qiashredder column	2 min	14000 rpm	
	Remove column and cap tube	3 min	14000 rpm	
	Transfer supernatant to tube with 600 ul of 70% EtOH and mix	15s	9500 rpm	
3. Capture	Transfer 700 ul at time to RNEasy column	15s	9500 rpm	Discard flow through
	Add rest to Rneasy column	15s	9500	Discard flow
	Add 700 ul Buffer RW1	15s	9500	
	Transfer column to new 2 ml collect tube			
	Add 500 ul buffer RPE	15s	9500	Discard flow
	Add another 500 ul buffer RPE	2 min	9500	Discard flow
	Additional spin to dry	1 min	14000	
4. Elute	Transfer to clean 1.5 ml tube			
	Add 30 ul RNAse free H2O	1 min	9500	
	Add another 30 ul RNAse free H2O	1 min	9500	Quantify