Total RNA extraction with RNeasy (QIAGEN)

0) Add 10 μL of β-ME to RLT (per 1 mL)
Add 44 mL of EtOH to conc. RPE
Set Dry-bath temp. at 56 °C
1) Measure material amount (< 100 mg)
2) Add 200 μL of RLT
3) Grind thoroughly with Hand-homogenizer
4) Add 250 μL of RLT
5) Vortex vigorously
6) Keep 4°C
7) Incubate 56 °C, 2 min
8) Transfer lysate to QIA shredder Spin Column (lilac)
9) Cfg. 2 min at max speed
10) Take sup and pour to new 2.0 mL tube
11) Add 225 μL of EtOH and mix immediately with pipette
12) Apply all sample to RNeasy Mini Column (pink)
13) Cfg. 15 sec at 10000 rpm
14) Discard the flow-through
15) Add 700 μL of RW1 to RNeasy column
16) Cfg. 15 sec at 10000 rpm
17) Discard the flow-through
18) Transfer RNeasy column to new 2.0 mL tube
19) Add 500 μL of RPE onto RNeasy column
20) Cfg. 15 sec at 10000 rpm
21) Discard the flow-through
22) Add another 500 μL of RPE onto RNeasy column
23) Cfg. 2 min at 10000 rpm to dry up
24) Transfer RNeasy column to new 1.5 mL tube
25) Cfg. 1 min at max speed
26) Transfer RNeasy column to new 1.5 mL tube
27) Add 30 μL RNase-free water onto RNeasy column
28) Cfg. 1 min at 10000 rpm
29) Repeat 26)-27) with first-elute
30) Measure RNA amount with NanoDrop
31) Keep -80 °C