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RNA Isolation

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ABSTRACT

Real Time Polymerase Chain Reaction

ATTACHMENTS

[RT_PCR.docx](#)

OPEN ACCESS

DOI:

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Protocol status: Working
We use this protocol and it's working

Created: Aug 13, 2019

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PROTOCOL integer ID:
26891

1 ● Get plates, aspirate media

- 2 ● Add 2mL ice cold DPBS
- 3 ○ Aspirate DPBS
- 4 ● Add 500uL Trizol/well in chemical hood
- 5 ● Bring yellow tubes to hood, collect trizol and add to tubes
- 6 ● Add 100uL chloroform to tubes
- 7 ○ hand shake 15s
- 8 ○ Place on ice 5min until see layers (aq/lipids/bottom)
- 9 ● Spin full speed 20min cold centrifuge
- 10 ○ During, make 70% ethanol on ice (3mL RNase-free water + 7mL ethanol)

- 11 ☐ Make new ep set 1-9
- 12 ☒ In hood, collect top layer after spinning and place into eppendorfs
- 13 ☐ **take note of first volume and make all the same
- 14 ☐ Mix 1:1 ethanol with supernatant, use same tip to transfer from eppendorfs into pink spin tubes
- 15 ☒ Spin 9000x 1min bench centrifuge
- 16 ☐ Discard flowthrough in hood, keep tube
- 17 ☒ Add 350uL RW1 buffer to tubes
- 18 ☐ Spin 1 min

- 19 ☐ During, prepare DNase soln (with syringe)
- 20 ● Add 70uL DNase soln to middle of each column, leave 15min RT
- 21 ● Repeat RW1 and spin
- 22 ☐ Discard collection tube and get new
- 23 ● Wash RPE 500uL
- 24 ☐ Spin 1min
- 25 ☐ Discard collection and keep tubes
- 26 ● Repeat RPE, spin 2min

☐ Discard tubes

27

28 ● Spin 1min empty to dry

29 ○ Get new eppendorfs, label

30 ● Place pink tubes on top of eppendorfs

31 ● Add 30uL RNase free water, centrifuge 1min

32 ○ Close tubes as quickly as possible and keep on ice→ nanodrop