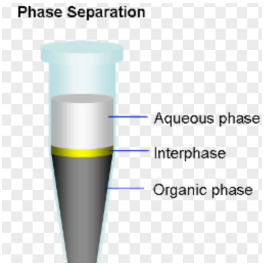
Aug 14, 2019

RNA Isolation



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

Created: August 14, 2019

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Abstract

Real Time Polymerase Chain Reaction

Attachments

RT PCR.docx

9KB

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 9000× 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

27 ○ Discard tubes

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