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cDNA protocol - Thermo Scientific RevertAid RT

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ABSTRACT

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Prepare workspace

1 Thaw RNA and reagents on ice. Label four foil lids: one to replace RNA lid, one for reaction and two for aliquots

2 UV sterilize 3 RNase free PCR plates, 8 boxes 10uL filter tips, and DEPC treated H_2O

3 Once thawed, flash spin down RNA in plate centrifuge and spin down reagents in mini centrifuge

cDNA conversion

4 Add **1 µL** RNA (**.1 ng** - **5 µg**) to empty PCR plate

5 Random Hexamer Primer **Thermo**

Add **1 µL** **Fisher Catalog #SO142**

Random Hexamer Primer **Thermo**

If not using **Fisher Catalog #SO142** protocol will be different. See product handbook RevertAid RT Reverse Transcription Kit **Thermo** **Fisher Catalog #K1691**

6 Add **10 µL** DEPC treated H_2O

If used amount other than **1 µL** RNA (step 1), adjust H_2O volume to bring total reaction volume to **12 µL**

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7 Add **4 µL** 5X reaction buffer

8 Add **1 µL** RiboLock RNase Inhibitor **Thermo Scientific Catalog #EO0381** (20 U/uL)

9 Add **2 µL** dNTP Mix (10 mM each) **Thermo Fisher Scientific Catalog #R0191**

10 Add **1 µL** RevertAid Reverse Transcriptase (200 U/µL) **Thermo Fisher Catalog #EP0441**

Total reaction volume should be **20 µL**

11 Mix gently with pipette and centrifuge briefly on "short"

12 Place in thermocycler and run program "cDNA"

Thermocycler cDNA program: Lid 105°C. 25°C 5 min, 42°C 60 min, 70°C for 5 min, ∞ hold 4°C.

13 Aliquot product into microplates and seal with labeled foil lid

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14 Store at -20 °C for up to 1 week or -80 °C for longer term storage

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