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High-Capacity cDNA Reverse Transcription

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We use this protocol and it's working

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Abstract

This protocol details the high-capacity cDNA reverse transcription.

Materials

- Applied BiosystemsTM High-Capacity cDNA Reverse Transcription Kit **Applied Biosystems (ThermoFisher Scientific) Catalog #**4368814

Master Mix:

	A	B
	Component	Volume
	1) Nuclease-free Water	3.2 µL
	2) 10X RT Buffer	2.0 µL
	3) 10X Random Primers	2.0 µL
	4) RNase Inhibitor	1.0 µL
	5) 25X dNTP Mix	0.8 µL
	6) MultiScribe Reverse Transcriptase	1.0 µL ***add last!!
	TOTAL per reaction	10 µL

Troubleshooting

Nanodrop

1 Nanodrop each isolated sample of RNA.

1.1 A good concentration of RNA is between 200 µL - 2000 µL .

- If the concentration is above 2000 µL then dilute the sample with water for a final concentration below 2000 µL .

1.2 A good 260/280 value is ~2.0.

1.3 A good 260/230 value is ~2.0-2.2.

cDNA Calculations

2 cDNA for all brain regions is made with 2000 ng of RNA. cDNA for isolated cells is made with the highest amount of RNA that can be made from the least concentrated sample.

TV per reaction = 20 µL (10 µL of RNA/Water + 10 µL of Master Mix)

- Calculate the RNA amount needed to make 2000 ng of RN for each sample

Ex. For a RNA concentration of 1250.0 µL .
2000/1250 = 1.6 µL of RNA

3 Calculate the amount of water to be added to the RNA for a TV = 10 µL

Ex. For 1.6 µL of RNA
10 µL – 1.6 µL of RNA = 8.4 µL of Water

Sample Number	RNA (ng/uL	260/280	260/230	Sample #	cDNA (2000 ng)	Water
1	849.1	2.11	2.34	1	2.36	7.64
2	1038.6	2.1	2.24	2	1.93	8.07
3	604.8	2.07	2.21	3	3.31	6.69
4	985.7	2.1	2.32	4	2.03	7.97
5	948.8	2.09	2.32	5	2.11	7.89
6	736.2	2.08	2.26	6	2.72	7.28
7	1185.5	2.1	2.33	7	1.69	8.31
8	450.5	2.12	2.19	8	4.44	5.56
9	1070.6	2.1	2.32	9	1.87	8.13
10	1000.9	2.11	2.33	10	2.00	8.00

Making cDNA

4 Thaw the isolated RNA and the following components of the High-Capacity cDNA reverse transcription kit On ice .

- 10X RT Buffer
- 25X dNTP Mix ([M] 100 millimolar (mM))
- 10X Random Primers – Can thaw at Room temperature
- RNase Inhibitor

Note

- **DO NOT thaw MultiScribe Reverse Transcriptase – it does not freeze at -20 °C and is prone to denaturing at higher temperatures. Keep at -20 °C until creating your master mix in below.**

5 While the above components thaw On ice pipette the calculated amount of water (from of cDNA calculations) to PCR tubes. This step can be done at Room temperature .

6 Place the PCR tubes with water On ice and then add the calculated amount of RNA (from of cDNA calculations) to its respective PCR tube.

7 Create the following master mix On ice . Make enough master mix for each sample plus a little extra (if you have 10 samples, make enough master mix for 11).

7.1 ▪ Add reagents to a 1.5 mL tube in the following order.

	A	B
	Component	**Volume**
	1) Nuclease-free Water	3.2 µL
	2) 10X RT Buffer	2.0 µL
	3) 10X Random Primers	2.0 µL
	4) RNase Inhibitor	1.0 µL
	5) 25X dNTP Mix	0.8 µL
	6) MultiScribe Reverse Transcriptase	1.0 µL ***add last!!
	TOTAL per reaction	**10 µL**

7.2 Mix gently.

8 Add 10 µL of master mix to each PCR tube On ice .

9 Mix PCR tubes gently then spin down briefly.

10 Keep On ice until performing the reverse transcription.

Perform Reverse Transcription

11 Place PCR tubes into the thermal cycler.

12 Set the Reaction Volume to 20 µL .

13 Set the following conditions:

Settings	Step 1	Step 2	Step 3	Step 4
Temp.	25°C	37°C	85°C	4°C
Time	10 minutes	120 minutes	5 minutes	Hold

14 Start the thermal cycler run.

15 When the samples reach take the PCR tubes out and store at 4 °C for short term use and at -20 °C for long term use.

Protocol references

Refer to the applied biosystems “High Capacity cDNA Reverse Transcription Kit User Guide” for reference.