Quick-Start Protocol October 2021

EZ2® AdnaTest CTC Select

Store the Reagent Cartridge EZ2 AdnaTest CTC Select, CTC Select Beads, and Oligo $(dT)_{25}$ Beads at 2–8°C upon arrival. The components must not be used beyond the expiration date, which is encoded in the barcode of the Q-Card.

Further information

- *EZ2 AdnaTest CTC Select Kit Handbook*: **www.qiagen.com/HB-2982**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **www.support.qiagen.com**

Notes before starting

- The EZ2 Connect Tip Rack - Large Volume (cat. no. 9027011), and the EZ1 filter-tips and holders (cat. no. 994900) are needed to run the protocol. They are not part of the instrument or the kit and need to be ordered separately.
- For cDNA generation, the Sensiscript RT Kit (cat. no. 205211) is needed for reverse transcription of the mRNA and needs to be ordered separately.

Procedure

1. Resuspend CTC Select Beads thoroughly (do not vortex) and transfer 100 µl for each blood sample into a 1.5 ml reaction tube.
2. Wash CTC Select Beads with 3 x 1 ml PBS using an AdnaMag-S rack.
3. Resuspend CTC Select Beads in the original volume (100 µl per blood sample).
4. When using standard EDTA tubes, transfer 5 ml whole blood into a 7 ml Large Volume Tube (provided). Alternatively, ACD-A blood can be used; transfer 5 ml ACD-A blood into a 7 ml Large Volume Tube and add 94 µl EDTA (0.5 M, pH 8.0).
5. Add 100 µl of washed CTC Select Beads to each blood sample.

6. Incubate for 30 min at room temperature under tilting and rotation at approximately 5 rpm.
7. Meanwhile, load reagent cartridges into the cartridge rack and allow to equilibrate to room temperature.
8. Remove the cap from an empty 1.5 ml screw cap tube (provided) and place in position 11 of the cartridge (refer to the handbook for an illustration of the EZ2 AdnaTest CTC Select cartridge).
9. Thoroughly resuspend the Oligo $(dT)_{25}$ Beads and pipet 20 µl into the bottom of position 12 of the reagent cartridge.
10. Open the instrument hood, load the cartridge rack, and place it into the EZ2 instrument.
11. Prepare the EZ2 Connect Tip Rack - Large Volume as follows (refer to the handbook for an illustration of the EZ2 Connect Tip Rack):
    - Position A: Tip holder with Filter Tip
    - Position B: Sample in Large Volume Tube from step 6
    - Position C: Empty large volume tube (7 ml, provided)
    - Position D: Tip holder with Filter Tip
12. Place the tip rack into the EZ2 Connect instrument.
13. Start the EZ2 instrument by following the instructions on the instrument's display.
14. After completion of the protocol on the EZ2 instrument, proceed without delay. Remove the 1.5 ml screw cap tube from position 11 and transfer the entire volume (500 µl) to a fresh 1.5 ml reaction tube (provided).
15. Place it on a AdnaMag-S rack. Wait for 1 min and carefully remove the supernatant.
16. Remove the magnet from the AdnaMag-S rack and resuspend beads in 29.5 µl RNase-free H_2O.
17. Incubate the beads at 50°C for 5 min. Then, place on ice immediately for 2 min.
18. Proceed with reverse transcription within 5 min; See Tables 1 and 2.

Table 1. Reverse transcription reaction setup

Component	Volume
RT Master Mix	
10x Buffer RT	4.0 µl
dNTP Mix (5 mM each dNTP)	4.0 µl
RNase inhibitor, 40 U/µl (Promega)	0.5 µl
Sensiscript Reverse Transcriptase	2.0 µl
Template RNA* mRNA/bead complex or RNase free H_2O	29.5 µl
Total volume	**40.0 µl**

* As RT control add 29.5 µl of RNase-free water instead of mRNA/bead complex. The volume of the mRNA/bead complex may vary slightly. Always use the total volume of this in the reverse transcription reaction.

Table 2. Reverse transcription program

Step	Time	Temperature
Reverse transcription	60 min	37°C
Denaturation	5 min	93°C
Cooling	∞	4°C

19. cDNA/bead complex can be used directly for downstream analyses, or stored at –30 to –15°C.

Document Revision History

Date	Changes
10/2021	Initial release

Scan QR code for handbook.

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