Quick-Start Protocol February 2018

AdnaTest ProstateCancerPanel AR-V7

Part 1: Isolation of mRNA and reverse transcription

AdnaPanel Prostate AR-V7 (cat. no. 396132; box 3), containing the AdnaPanel PrimerMixes, AdnaPanel Positive Control, Internal Control and Inhibition Control, must be stored at –30 to –15°C. Aliquot the primer mix to prevent possible contamination and repeated temperature changes. The components must not be used beyond the expiration date.

Further information

- *AdnaTest ProstateCancerPanel AR-V7 Handbook*: **www.qiagen.com/HB-2525**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: Toll-free 00800-22-44-6000 or **support.qiagen.com**

Protocol

1. Equilibrate AdnaTest Lysis/Binding Buffer and RNA Purification Buffers A and B to room temperature and place Tris·HCl Buffer on ice.
2. Wash Oligo(dT)$_{25}$ Beads (20 µl per sample) twice with AdnaTest Lysis/Binding Buffer (20 µl per sample).
3. Add 2 µl AdnaPanel Inhibition Control to each sample (200 µl lysate from the AdnaTest ProstateCancerSelect enrichment of tumor cells).
4. As a non-sample reference, pipet 200 µl AdnaTest Lysis/Binding Buffer into a new reaction tube and add 2 µl AdnaPanel Inhibition Control. Process this Inhibition Control Sample as a cell lysate sample.
5. Add 20 µl washed Oligo(dT)$_{25}$ Beads to each sample.

6. Incubate for 10 min at room temperature under tilting and rotation at approximately 5 rpm.
7. Place the reaction tube in the AdnaMag-S and remove supernatant.
8. Wash beads with 2 x 100 µl RNA Purification Buffer A.

   Important: To avoid any loss of beads, rinse lid and tube wall thoroughly.
9. Resuspend beads in 100 µl RNA Purification Buffer B and transfer into a new 1.5 ml tube (provided).
10. Wash beads with 1 x 100 µl RNA Purification Buffer B.
11. Wash beads with 1 x 100 µl ice-cold Tris·HCl Buffer.
12. Resuspend beads in 14.75 µl RNase-Free Water.
13. Incubate for 5 min at 65°C and place on ice for at least 2 min.
14. Continue with reverse transcription (Table 1 and Table 2).

Table 1. Reverse transcription reaction setup

Component	Volume
RT master mix	
10x Buffer RT	2.0 µl
dNTP Mix (5 mM each dNTP)	2.0 µl
RNase inhibitor, 40 U/µl (Promega)	0.25 µl
Sensiscript® Reverse Transcriptase	1.0 µl
Template RNA*	
mRNA/bead complex or RNase-free water	14.75 µl
Total volume	**20 µl**

* As an RT control, add 14.75 µl 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

15. Proceed with "Preamplification PCR and qRT-PCR" in Quick-Start Protocol Part 2 or store cDNA at –20°C for a maximum of 4 weeks.

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