Quick-Start Protocol February 2018

AdnaTest ProstateCancerPanel AR-V7

Part 2: Preamplification PCR and qRT-PCR

AdnaPanel Prostate AR-V7 (cat. no. 396132; box 3), containing the AdnaPanel PrimerMixes, AdnaPanel Positive Control, Internal Control and Inhibition Control, must be stored separately 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. Perform preamplification PCR (Table 1 and Table 2).

Table 1. Preparation of the preamplification PCR

Component	Volume
Preamplification PCR master mix	
2x Multiplex PCR Master Mix	25 µl
RNase-Free Water	13.75 µl
AdnaPanel PrimerMix PreAmp AR-V7	5 µl
cDNA or RT Negative Control	6.25 µl
Total volume	**50 µl**

Table 2. Preamplification PCR cycling program

	Temperature	Time
Initial activation step	95°C	5 min
3-step cycling		
Denaturation	95°C	30 s
Annealing	60°C	90 s
Extension	72°C	90 s
Number of cycles	**18**	

2. Freeze the samples immediately at –20°C after completion of the last cycle. Wait for a minimum of 15 min to allow the samples to cool down before continuing with dilution of the samples.
3. Spin down beads and dilute each sample 1:10 by mixing 20 µl preamplified cDNA (without beads) and 180 µl RNAse/DNAse-free water in a new reaction tube.

   Important: Do not dilute AdnaPanel Internal Control and Positive Control.

Table 3. Preparation of the qRT-PCR master mix for seven analyses

Component	Volume
2X miRCURY SYBR® Green Master Mix	52.5 µl*
RNase-free water	0–10.5 µl†
ROX™ reference dye	0–10.5 µl†
Preamplified sample (dilution 1:10) or AdnaPanel Positive Control AR-V7 or RT Negative control	21 µl
Total volume qRT-PCR master mix	**84 µl**

* Pay attention to the viscosity of SYBR Green during pipetting.

† Depending on the instrument; total amount of ROX and RNAse/DNAse-free water is 1.5 µl.

Table 4. qRT-PCR setup per reaction

Component	Volume
qRT-PCR master mix (prepared as per Table 3)	12 µl
AdnaPanel PrimerMix CD45 or AdnaPanel PrimerMix GAPDH or AdnaPanel PrimerMix PSA or AdnaPanel PrimerMix PSMA or AdnaPanel PrimerMix AR or AdnaPanel PrimerMix AR-V7 or AdnaPanel PrimerMix Inhibition Control	3 µl
Total volume	**15 µl**

Table 5. Preparation of the qRT-PCR master mix (AdnaPanel Internal Control and AdnaPanel Inhibition Control)

Component	Volume
2X miRCURY SYBR Green Master Mix	7.5 µl*
RNase-free water	0–1.5 µl†
ROX reference dye	0–1.5 µl†
AdnaPanel PrimerMix IC or AdnaPanel PrimerMix Inhibition Control	3.0 µl
AdnaPanel Internal Control or AdnaPanel Inhibition Control	3.0 µl
Total volume	**15.0 µl**

* Pay attention to the viscosity of SYBR Green during pipetting.

† Depending on the instrument; total amount of ROX and RNAse/DNAse-free water is 1.5 µl.

Table 6. qRT-PCR cycling program

	Temperature	Time
Initial activation step	95°C	10 min
3-step cycling		
Denaturation	95°C	10 s
Annealing	60°C	10 s
Extension and data collection	78°C	10 s
Number of cycles	**35**	
Melt curve	**60–95°C**	

4. Data collection of SYBR Green signal and melting curve measurement at 78°C.

For evaluation of the results, please refer to the *AdnaTest ProstateCancerPanel AR-V7 Handbook*.

IMPORTANT: If the protocol is not followed exactly, this may result in false-negative or false-positive results.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.