April 2020

AdnaTest ProstateCancerSelect and ProstateCancerDetect Handbook

For enrichment of tumor cells from whole blood in prostate cancer research and detection of prostate-cancer-associated gene expression in enriched tumor cells

REF

395032 (AdnaTest ProstateCancerSelect)
396032 (AdnaTest ProstateCancerDetect)

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Sample to Insight

QIAGEN

Contents

Kit Contents

AdnaTest ProstateCancerSelect	
Catalog no.	**395032**
Number of tests	**12**
Collection Tubes (1.5. ml)	3 x 5
Collection Tubes (15 ml)	24
ProstateSelect Beads	1.2 ml
AdnaTest Lysis/Binding Buffer	2 x 1.2 ml
Quick-Start Protocol	1

AdnaTest ProstateCancerDetect	
Catalog no.	**396032**
Number of tests	**12**
AdnaTest RNA Reagent	**Box 1**
AdnaTest Lysis/Binding Buffer	2 ml
Oligo(dT)$_{25}$ Beads	355 µl
RNA Purification Buffer A	4 ml
RNA Purification Buffer B	4 ml
Tris-HCL Buffer	2 ml
AdnaTest ProstateCancerDetect	**Box 2**
AdnaTest PrimerMix ProstateDetect	144 µl
AdnaTest Positive Control Prostate (C+)	40 µl
AdnaTest PrimerMix AR-Detect	144 µl
AdnaTest Positive Control AR (C+)	40 µl
Quick-Start Protocol	1

The AdnaTest ProstateCancerDetect reagents are sufficient to analyze 6 PCR controls and 12 blood samples.

Shipping and Storage

The AdnaTest ProstateCancer system is delivered in 3 boxes.

AdnaTest ProstateCancerSelect (cat. no. 395032) and the AdnaTest RNA Reagent Box 1 (Box 1 of cat. no. 396032) must be stored at 2–8°C. The components must not be used beyond the expiration date.

AdnaTest ProstateCancerDetect Box 2 (Box 2 of cat. no. 396032), containing the AdnaTest PrimerMixes and AdnaTest Positive Controls, must be stored in a constant-temperature freezer at –30 to –15°C. To prevent possible contamination and repeated temperature changes, aliquot the primer mix. The components must not be used beyond the expiration date.

Intended Use

AdnaTest ProstateCancerSelect and AdnaTest ProstateCancerDetect are for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of these products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Abbreviations

AdnaMag-L	magnetic particle concentrator (large)
AdnaMag-S	magnetic particle concentrator (small)
AR	androgen receptor
bp	base pairs
C+	positive control
C–	negative control
cDNA	complementary deoxyribonucleic acid
DNA	deoxyribonucleic acid
dNTPs	deoxynucleotide triphosphates
EGFR	epidermal growth factor receptor
kb	kilobases
mRNA	messenger ribonucleic acid
PCR	polymerase chain reaction
PSA	prostate-specific antigen
PSMA	prostate-specific membrane antigen
RNase	ribonuclease
rpm	revolutions per minute
RT	reverse transcription

Symbols

Use by

Temperature limitation

Catalog number

Manufacturer

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of AdnaTest ProstateCancerSelect and AdnaTest ProstateCancerDetect is tested against predetermined specifications to ensure consistent product quality.

Introduction

The AdnaTest ProstateCancer system is used for the enrichment and molecular characterization of circulating tumor cells (CTCs) from whole blood in prostate cancer research: The AdnaTest ProstateCancerSelect is used for enriching CTCs from whole blood, while the AdnaTest ProstateCancerDetect is subsequently used for analysis of prostate-cancer-associated gene expression. The specificity of the detection is at least 90%. In spiking experiments, 5 tumor cells in 5 ml of whole blood are detected at a recovery rate of at least 90%.

Successful CTC detection is based on the combination of combinations principle (COCP). Each AdnaTest has a unique combination of tumor-associated markers and an optimized combination of antibodies for cell selection. By combining a highly specific immunomagnetic cell-selection system using an optimized antibody combination with highly sensitive RT-PCR technology using a combination of mRNA tumor markers, the highest degrees of specificity and sensitivity can be expected. The AdnaTest uses a 2-step process (select and detect) to generate results within 5 hours.

AdnaTest ProstateCancerSelect

AdnaTest ProstateCancerSelect enables the immunomagnetic enrichment of tumor cells via epithelial and tumor-associated antigens. Antibodies against epithelial and tumor-associated antigens are conjugated to magnetic beads for labeling of tumor cells in whole blood. Labeled cells are extracted by a magnetic particle concentrator (AdnaMag-L and AdnaMag-S) and are subsequently lysed (Figure 1 and Figure 2).

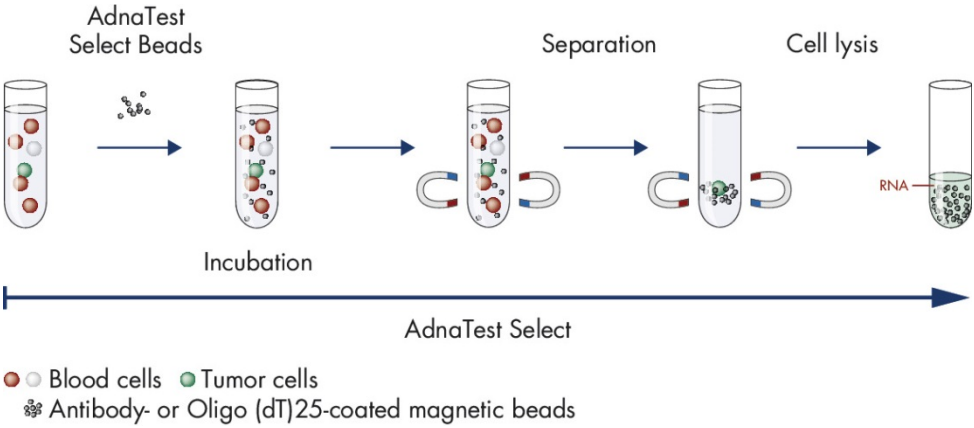

Figure 1. AdnaTest ProstateCancerSelect: Immunomagnetic cell selection with multiple tumor-associated antibodies. In the first step, the CTCs in the blood are enriched (AdnaTest Select). This is achieved using antibody-coated magnetic particles (beads). Several antibodies are used, which bind with high specificity and affinity to the corresponding cancer cells. The enriched cells are lysed and subsequently purified several times to extract mRNA.

The cell lysate is used for further analysis with AdnaTest ProstateCancerDetect.

AdnaTest ProstateCancerDetect

AdnaTest ProstateCancerDetect contains Oligo $(dT)_{25}$ Beads for the isolation of mRNA from the lysate of enriched tumor cells. Reverse transcription results in cDNA, which is subsequently used as template for tumor-cell detection and characterization by multiplex PCR. The AdnaTest PrimerMix ProstateDetect allows amplification of 3 tumor-associated antigens and 1 control gene. The AdnaTest PrimerMix AR-Detect allows amplification of the androgen receptor (AR).

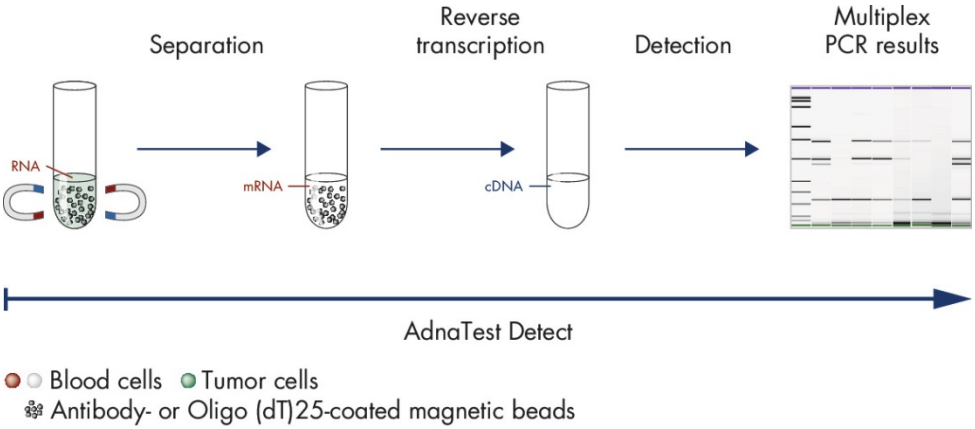

Figure 2. AdnaTest ProstateCancerDetect: Multiplex PCR of various cancer-associated tumor markers. In a second step, the enriched cells are examined by RT-PCR for tumor-associated expression patterns. The mRNA strands are reverse transcribed into cDNA. Subsequently, several associated tumor markers can be amplified using multiplex PCR and visualized.

The primers generate fragments of the following sizes:

PrimerMix ProstateDetect

- PSMA: 449 bp
- PSA: 357 bp
- EGFR: 163 bp
- Actin: 120 bp (internal PCR control)

PrimerMix AR-Detect

- AR: 440 bp

Note: Fragment sizes may vary slightly. Make sure to use the AdnaTest Positive Controls for assignment of the detected signals.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

AdnaTest ProstateCancerSelect

Equipment

- Tube rotator for 15 ml and 1.5 ml tubes (e.g., ELMI Ltd. cat. no. IMIX-03)
- Magnetic particle concentrators
  - AdnaMag-L (cat. no. 399921)
  - AdnaMag-S (cat. no. 399911)

Material

- AdnaTube Tubes (cat. no. 399932), when working with BD Vacutainer® ACD-A Tubes (Becton Dickinson GmbH cat. no. 366645 [EU]; 364606 [US])
- Sterile, RNase-free 10 ml glass or plastic pipettes and pipettor
- Sterile, RNase-free 1.5 ml reaction tubes (e.g., Sarstedt cat. no. 72.690)
- Pipettes and RNase-free pipette tips with aerosol barrier, suitable for pipetting volumes from 100 µl to 1000 µl

Reagents

- Phosphate buffered saline (PBS), pH 7.0–7.3 (e.g., Fisher cat. no. VX14190169, D-PBS)

AdnaTest ProstateCancerDetect

Equipment

- Tube rotator for 1.5 ml tubes
- Magnetic particle concentrator AdnaMag-S
- Thermal block or water bath (65°C)
- Thermal cycler with a heated lid and a heating rate of 2°C/s.
- Analysis system, such as the Agilent® 2100 Bioanalyzer (Agilent Technologies)

Material

- Sterile, RNase-free thin-wall 0.2 ml PCR tubes
- Sterile, RNase-free 1.5 ml reaction tubes
- Pipettes and RNase-free pipette tips with aerosol barrier, suitable for pipetting volumes from 1 µl to 200 µl

Reagents

- Sensiscript® RT Kit (cat. no. 205211, 50 reactions)

  Note: The Sensiscript RT Kit is sufficient for only 25 samples because twice the volume is required for each reaction.
- Recombinant RNasin®, RNase-inhibitor, 2500 U (Promega cat. no. N2511)
- HotStarTaq® Master Mix Kit (cat. no. 203443, 250 U)
- Crushed ice

Important Notes

Sample preparation

- Blood samples must be taken before the application of therapeutic substances. Do not use the AdnaTest ProstateCancerSelect earlier than 7 days after the last therapeutic intervention.
- Blood collection: If sample transportation is less than 4 hours, use tubes containing EDTA as anticoagulant (e.g., S Monovette® K3 EDTA, Sarstedt cat. no. 01.1605.001) to draw at least 7.5 ml of whole blood.
- If sample transportation is longer than 4 hours, use BD Vacutainer ACD-A Tubes to draw at least 8.5 ml of whole blood. Before further processing using the AdnaTest, 5 ml ACD-A blood must be transferred into an AdnaTube.
- Blood must be stored at 2–8°C immediately.
- Samples should be processed as soon as possible but not later than 4 hours after blood withdrawal (when using standard EDTA tubes) or within 30 hours (when using BD Vacutainer blood collection tubes in combination with AdnaTubes).
- The blood sample must not be hemolyzed.

Handling

- ProstateSelect Beads contain sodium azide as preservative. Sodium azide is cytotoxic and must, therefore, be removed before using the beads. (See “Protocol: Enrichment of Tumor Cells Using AdnaTest ProstateCancerSelect”, page 14.)
- All components and additional reagents provided by other suppliers must be stored according to their instructions. Follow the safety advice of the respective manufacturers.
- Wear protective gloves to avoid contamination with DNA, RNA, and RNases.
- Aliquot the ProstateSelect Beads to avoid contamination.

- The test must be performed in the denoted sequence and must comply with all specifications stated in respect of incubation times and incubation temperatures.
- Discard samples if the selection beads agglutinate during cell enrichment.
- Perform sample processing, including reverse transcription and subsequent analysis of amplified PCR products, in different rooms, if possible, to avoid cross-contamination.
- The use of products from suppliers other than those suggested may adversely affect the results.
- Observe the safety and hygiene regulations of the laboratory (e.g., wear lab coats, protective goggles, and gloves).

Protocol: Enrichment of Tumor Cells Using AdnaTest ProstateCancerSelect

Important points before starting

- Before beginning the procedure, read "Important Notes" (page 12).
- It is necessary to remove sodium azide by washing the ProstateSelect Beads prior to use, as described below in "Procedure A: Preparation of the ProstateSelect Beads".
- Use the provided 1.5 ml collection tubes only for the protocol step indicated.

Things to do before starting

- Ensure that the AdnaTest Lysis/Binding Buffer is equilibrated to room temperature (15–25°C). If precipitate is observed, equilibrate the reagent to room temperature and mix until the precipitate is completely dissolved.

Procedure A: Preparation of the ProstateSelect Beads

1. Resuspend the ProstateSelect Beads thoroughly by pipetting.

   Important: Do not vortex.

2. Calculate the volume of ProstateSelect Beads required for all samples to be processed (100 µl per sample), and transfer the calculated volume into a 1.5 ml reaction tube (not provided).

   If more than 10 samples are to be processed, use additional 1.5 ml reaction tubes.

3. Place the tube into the AdnaMag-S rack.
4. After 1 min, remove the supernatant with a pipette.

   Important: Do not touch the beads when removing the supernatant.

5. Wash steps:
   5a. Remove the magnet slider from the AdnaMag-S rack.
   5b. Add 1 ml PBS and resuspend the beads by repeated pipetting.
   5c. Place the magnet slider into the AdnaMag-S rack.
   5d. After 1 min, remove the supernatant completely with a pipette.
   5e. Repeat steps 5a–5d twice (3 washes in total).

Procedure B: Selection of tumor cells

1. When using standard EDTA tubes, pipet 5 ml of a blood sample into a 15 ml collection tube.

   When using ACD-A blood in a BD Vacutainer ACD-A Tube, transfer 5 ml of blood into an AdnaTube.

   Note: AdnaTubes are mandatory when using BD Vacutainer ACD-A Tubes.
2. Resuspend the ProstateSelect Beads thoroughly (prepared in step 6 of Procedure A) by pipetting, and add 100 µl of these beads to each blood sample.
3. Rotate tubes slowly (approximately 5 rpm) for 30 min at room temperature on a device that allows both tilting and rotation.
4. Place tubes into the AdnaMag-L rack without the magnet slider. Swing the AdnaMag-L rack downwards to release blood droplets captured in the cap.
5. Insert the magnet slider and incubate the tubes in the AdnaMag-L rack for 3 min at room temperature.
6. Remove the supernatant completely with a 10 ml pipette without touching the beads.

   Important: Do not touch the beads when removing the supernatant.

7. Wash steps:
   7a. Remove the magnet slider from the AdnaMag-L rack.
   7b. Add 5 ml PBS. Close the tubes and shake the AdnaMag-L rack gently back and forth 5 times to resuspend the magnetic bead/cell complexes.
   7c. Swing the AdnaMag-L rack with the tubes downward twice to release droplets captured in the cap.
   7d. Place the magnet slider into the AdnaMag-L rack and incubate for 1 min at room temperature.
   7e. Remove supernatant completely with a pipette.
   7f. Repeat steps 7a–7e twice (3 washes in total).
8. Remove the magnet slider from the AdnaMag-L rack.
9. Resuspend the magnetic bead/cell complexes in 1 ml PBS and transfer each sample into a 1.5 ml reaction tube (not provided).
10. Place reaction tubes into the AdnaMag-S rack with an inserted magnet slider.

    Note: The magnet slider of the AdnaMag-S rack can be inserted in 2 positions. Always insert the slider the white plastic film facing forward to make sure that the magnets are next to the reaction tubes.
11. After 1 min, remove the supernatant completely with a pipette to optimize the following cell lysis.
12. Remove the magnet slider from the AdnaMag-S rack.
13. Add 200 µl AdnaTest Lysis/Binding Buffer (equilibrated to room temperature) to each reaction tube. Resuspend by pipetting at least 5 times.
14. Insert the magnet slider into the AdnaMag-S rack, and incubate for 1 min.
15. Transfer supernatant (cell lysate) into new 1.5 ml reaction tubes (provided).
16. Discard the tubes that contain the beads.
17. Continue with mRNA isolation (see "Protocol: Detection of Prostate-Cancer-Associated Gene Expression in Enriched Tumor Cells Using AdnaTest ProstateCancerDetect", page 17) immediately, or store the cell lysates at –30 to –15°C for up to 2 weeks.

Protocol: Detection of Prostate-Cancer-Associated Gene Expression in Enriched Tumor Cells Using AdnaTest ProstateCancerDetect

Important points before starting

- Before beginning the procedure, read “Important Notes” (page 12).
- Procedures A–C describe the isolation of mRNA and reverse transcription.
- Use the provided 1.5 ml collection tubes only for the protocol step indicated.

Things to do before starting

- Ensure that AdnaTest Lysis/Binding Buffer is equilibrated to room temperature. If precipitate is observed, equilibrate the reagent to room temperature and mix until the precipitate is completely dissolved.
- Equilibrate RNA Purification Buffer A and RNA Purification Buffer B to room temperature. Place Tris-HCL Buffer on ice.
- Thaw 10x Buffer RT and dNTPs, from the Sensiscript RT Kit, at room temperature. Mix by vortexing. Centrifuge briefly and store on ice. Thaw RNase-free water (part of the Sensiscript RT Kit).
- Adjust a thermal block or water bath to 65°C.

Procedure A: Preparation of Oligo(dT)$_{25}$ Beads

1. Resuspend the Oligo(dT)$_{25}$ Beads thoroughly by pipetting before use.

   Important: Do not vortex.

2. Calculate the volume of the beads required for all samples to be processed (20 µl per sample, plus 10%) and transfer the calculated volume into an RNase-free 1.5 ml reaction tube (not provided).

3. Place the tube into the AdnaMag-S rack.

   Note: The magnet slider of the AdnaMag-S rack can be inserted in 2 positions. Always insert the slider with the white plastic film facing forward to make sure that the magnets are next to the reaction tubes.

4. After 1 min, remove the supernatant with a pipette.

5. Wash steps:

   5a. Remove the magnet slider from the AdnaMag-S rack.

   5b. Add the original volume (step 2, page 17) AdnaTest Lysis/Binding Buffer and resuspend the beads by repeated pipetting. Resuspend gently to avoid foaming.

   5c. Insert the magnet slider into the AdnaMag-S rack.

   5d. After 1 min, remove the supernatant completely.

   5e. Repeat steps 5a–5d once (2 washes in total).

6. Remove the tube from the AdnaMag-S rack, and resuspend the beads in AdnaTest Lysis/Binding Buffer to the original volume (step 2, page 17). Proceed with "Procedure B: mRNA isolation".

Procedure B: mRNA isolation

1. Add 20 µl of Oligo(dT)$_{25}$ Beads (step 6, above) to each tube containing cell lysate (step 15, page 16).

2. Rotate tubes slowly (approximately 5 rpm) for 10 min at room temperature on a device that allows both tilting and rotation.

3. Place the tubes into the AdnaMag-S rack without the magnet slider. Swing the AdnaMag-S rack downwards to release beads and liquid captured in the cap.

4. Insert the magnet slider, wait for 1 min, and then remove the supernatant.

5. Wash steps 1:
    5a. Remove the magnet slider from the AdnaMag-S rack.
    5b. Add 100 µl RNA Purification Buffer A to each tube and resuspend the beads by repeated pipetting. To avoid any loss of beads, rinse lid and tube wall thoroughly.
    5c. Insert the magnet slider into the AdnaMag-S rack.
    5d. After 1 min, remove the supernatant completely.
    5e. Repeat steps 5a–5d once (2 washes in total).
6. Wash steps 2:
    6a. Remove the magnet slider from the AdnaMag-S rack.
    6b. Add 100 µl RNA Purification Buffer B to each tube. Resuspend the beads by pipetting, and transfer into new 1.5 ml reaction tubes (provided).
    6c. Insert the magnet slider into the AdnaMag-S rack.
    6d. After 1 min, remove the supernatant completely. This step must be carried out carefully (watch the pellet), because the beads may slide and could be removed by mistake.
    6e. Using the same reaction tubes, repeat steps 6a–6d once (2 washes in total).
7. Remove the magnet slider from the AdnaMag-S rack.
8. Add 100 µl ice-cold Tris-HCL Buffer to each tube, and resuspend the beads by pipetting.
9. Insert the magnet slider into the AdnaMag-S rack.
10. After 1 min, remove the supernatant completely.
11. Remove the magnet slider from the AdnaMag-S rack.
12. Resuspend the mRNA/bead-complex in 14.75 µl RNase-free water.
13. Transfer the tubes to a thermal block or water bath, and incubate for 5 min at 65°C.
14. Place the tubes on ice immediately for at least 2 min.
15. Continue immediately (within 5 min) with reverse transcription (Procedure C: Reverse transcription using the Sensiscript RT Kit).

    Important: Do not store the mRNA/bead complex.

Procedure C: Reverse transcription using the Sensiscript RT Kit

1. Prepare the RT Master Mix on ice. The RT Master Mix is prepared as shown in Table 1 according to the number of samples.

   The volume of the RT Master Mix should be 10% greater than what was calculated for the total number of reverse transcription reactions. A negative control reaction without addition of mRNA must always be prepared (RT control).

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	**20.0 µl**

* As RT control, add 14.75 µl RNase-free water instead of mRNA/bead-complex. The volume of the mRNA/bead-complex may vary slightly. In any case, use the total volume for reverse transcription.

2. Vortex the RT Master Mix. Centrifuge briefly, and pipet 5.25 µl for each reaction into 0.2 ml PCR tubes.
3. Resuspend the mRNA/bead complexes (step 12, page 19) carefully with a pipette. Transfer the total volume into the 0.2 ml PCR reaction tube containing the RT Master Mix. Mix thoroughly by repeated pipetting.

4. The cDNA is synthesized in a thermal cycler under the following conditions (Table 2).

Table 2. Reverse transcription program

Temperature	Time
37°C	60 min
93°C	5 min
4°C	∞

5. Place reaction tubes with the cDNA on ice, or store at –30 to –15°C for a maximum of 4 weeks.

   Continue with “Protocol: Multiplex PCR, Singleplex PCR, and Fragment Analysis”, page 22.

Protocol: Multiplex PCR, Singleplex PCR, and Fragment Analysis

Important point before starting

- Before beginning the procedure, read "Important Notes" (page 12).

Things to do before starting

- Thaw HotStarTaq Master Mix, AdnaTest PrimerMix ProstateDetect, AdnaTest Positive Control Prostate, AdnaTest PrimerMix AR-Detect, AdnaTest Positive Control AR, and RNase-free water. Vortex, centrifuge quickly, and store on ice.

Procedure A: Multiplex PCR (AdnaTest ProstateDetect)

1. Prepare the PCR Master Mix as shown in Table 3 according to the number of samples.

   The volume calculation of the PCR Master Mix should include at least 10% excess volume. Note that an AdnaTest Positive Control Prostate, RNase-free water as negative control, and the RT control must always be included.

Table 3. Preparation of the multiplex PCR

Component	Volume
Multiplex PCR Master Mix	
HotStarTaq Master Mix	12.5 µl
RNase-free water	4.5 µl
PrimerMix ProstateDetect	4.0 µl
cDNA *or* RT control *or* Negative control (RNase-free water) *or* Positive control (C+), each:	4.0 µl
Total volume	**25.0 µl**

2. For each preparation, dispense 21.0 µl of the PCR Master Mix into 0.2 ml PCR reaction tubes. Resuspend the cDNA/bead mix by pipetting, and add 4.0 µl of this to each reaction tube.

   Note: For negative control, add 4.0 µl of RNase-free water instead of cDNA.

3. Use a thermal cycler for the PCR, following the program described in Table 4. Run the thermal cycler with a ramp of 2°C/second. The PCR is performed with a total of 42 cycles.

Table 4. PCR cycling program

	Time	Temperature
Initial activation step	15 min	95°C
3-step cycling (42 cycles)		
Denaturation	30 s	94°C
Annealing	30 s	61°C
Extension	30 s	72°C
Final extension	10 min	72°C
Cooling	∞	4°C

Procedure B: Singleplex PCR (AdnaTest AR-Detect)

4. Prepare the PCR Master Mix as shown in Table 5 according to the number of samples.

   The volume calculation of the PCR Master Mix should include at least 10% excess volume. Note that an AdnaTest Positive Control, RNase-free water as negative control, and the RT control must always be included.

5. For each preparation, dispense 21.0 µl of the PCR Master Mix into 0.2 ml PCR reaction tubes. Resuspend the cDNA/bead mix by pipetting, and add 4.0 µl of this to each reaction tube.

   Note: For negative control, add 4.0 µl RNase-free water instead of cDNA.

Table 5. Preparation of the singleplex PCR

Component	Volume
Singleplex PCR Master Mix	
HotStarTaq Master Mix	12.5 µl
RNase-free water	4.5 µl
PrimerMix AR-Detect	4.0 µl
cDNA *or* RT control *or* Negative control (RNase-free water) *or* Positive control (C+), each:	4.0 µl
Total volume	**25.0 µl**

6. Use a thermal cycler for the PCR following the program described in Table 6. Run the thermal cycler with a ramp of 2°C/second. The PCR is performed with a total of 35 cycles.

Table 6. PCR cycling program

	Time	Temperature
Initial activation step	15 min	95°C
3-step cycling (35 cycles)		
Denaturation	30 s	94°C
Annealing	30 s	60°C
Extension	60 s	72°C
Final extension	10 min	72°C
Cooling	∞	4°C

Procedure C: Fragment analysis on the Agilent 2100 Bioanalyzer

Perform the analysis with the Agilent 2100 Bioanalyzer on a DNA 1000 LabChip®. Follow the instructions of the DNA 1000 LabChip manual and make sure that no beads are transferred into the LabChip. Magnetic beads in the gel may cause invalid results.

1. Start the Bioanalyzer software **2100 expert**. Select **Instrument** (under **Contexts**) and then click the **Assay** button (next to **Assay Selection**).
2. Select **Electrophoresis** > **DNA 1000 Series II.xsy**. Prepare the chip and start the run.
3. For evaluation of the results, set a detection threshold:
   3a. Under **Contexts**, select **Data** and then click the **Assay Properties** tab. Select **Global** and **Normal** from the drop-down menu on the right.
   3b. Select **Sample Setpoints** > **Integrator > height threshold (FU)** and set this value to **0** (default value is **20**) to detect all signals.

Analysis of the results for AdnaTest ProstateDetect

The test is considered positive if a PCR fragment of at least 1 tumor-associated transcript (PSMA, PSA, or EGFR) is clearly detected.

Using the Agilent 2100 Bioanalyzer, peaks with a concentration ≥ 0.10 ng/µl are positive (Figure 3).

The fragment of the control gene actin must be detected in all patient samples (internal PCR control). An actin signal provides a positive control for successful cell separation, reverse transcription and multiplex PCR. Negative control and RT control samples must not show any bands larger than 80 base pairs (primer–dimers).

A fragment larger than 900 bp indicates contamination with genomic DNA. The separation process was not successful and the results are invalid in this case.

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

If assistance is needed to interpret the results, please contact QIAGEN Technical Services at **support.qiagen.com**.

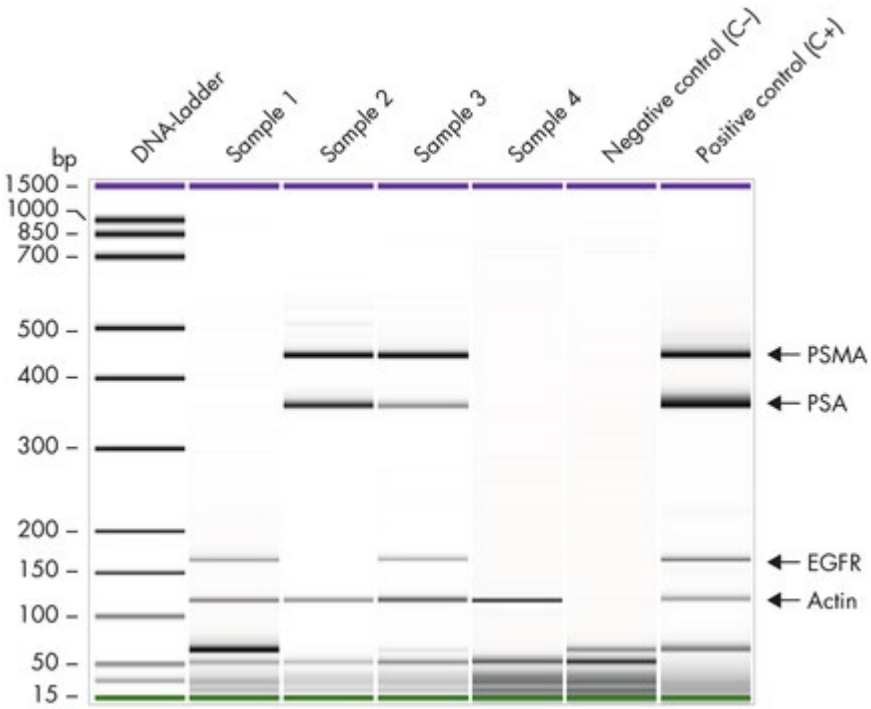

Figure 3. AdnaTest ProstateCancerDetect results of multiplex PCR samples analyzed with an Agilent 2100 Bioanalyzer. The first lane shows the DNA size standard (DNA-Ladder). Sample 1 is positive for EGFR, sample 2 is positive for PSMA and PSA, and sample 3 is positive for PSMA, PSA, and EGFR. Sample 4 is negative. Actin is detected in samples 1–4. The PCR negative (C–) and positive control (C+) are shown in the last 2 lanes.

Analysis of the results for AdnaTest AR-Detect

If you are using the Agilent 2100 Bioanalyzer, peaks with a concentration ≥0.15 ng/µl for AR are positive (Figure 4).

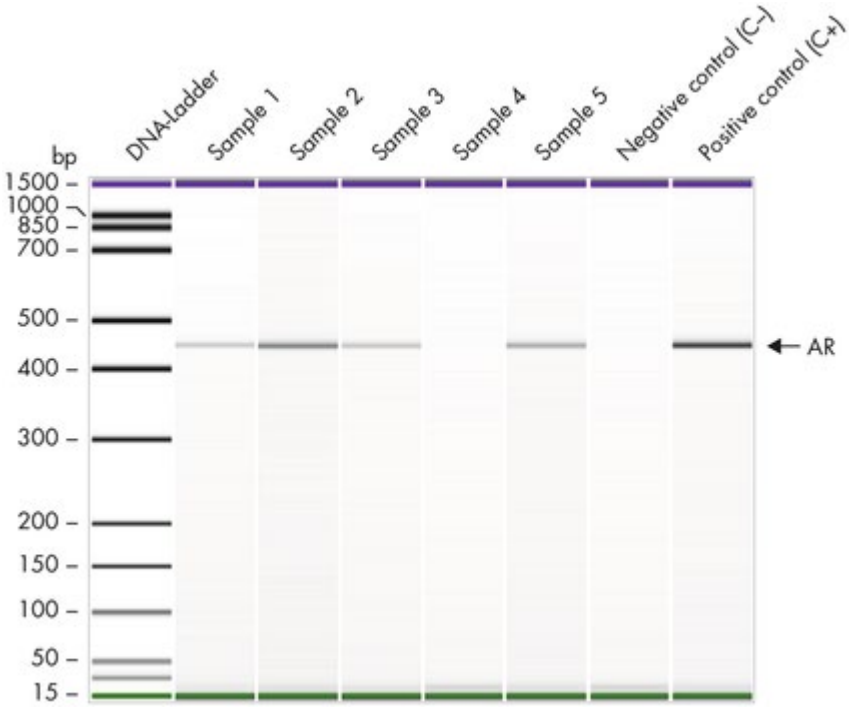

Figure 4. AdnaTest ProstateCancerDetect results of singleplex PCR samples. The first lane shows the DNA size standard (DNA-Ladder). Samples 1–3 and sample 5 are positive for AR. Sample 4 is negative. The PCR negative (C–) and positive control (C+) are shown in the last 2 lanes.

The fragment of the control gene actin must be detected in all patient samples (internal PCR control). An actin signal provides a positive control for a successful cell separation, reverse transcription, and singleplex PCR. The negative control and the RT control samples must not show any bands larger than 80 base pairs (primer–dimers).

A fragment larger than 900 bp indicates contamination with genomic DNA, suggesting that a problem occurred during cell separation. The results are invalid in this case.

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

If assistance is needed to interpret the results, please contact QIAGEN Technical Services at **support.qiagen.com**.

Troubleshooting guide

For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

Ordering Information

Product	Contents	Cat. no.
AdnaTest ProstateCancerSelect	For isolation of CTCs and the subsequent extraction of mRNA from human whole blood for 12 preparations	395032
AdnaTest ProstateCancerDetect	RT-PCR kit for detection of prostate-cancer-associated gene expression in enriched tumor cells	396032
Related products		
AdnaTube	12 sample tubes containing EDTA. Use only with anticoagulated blood collected in A-CDA blood collection tubes from BD	399932
AdnaMag-L	Magnetic rack for 8 tubes, 15 ml	399921
AdnaMag-S	Magnetic rack for 8 tubes, 1.5 ml	399911
Sensiscript RT Kit (50)	For 50 reverse-transcription reactions:* Sensiscript Reverse Transcriptase, 150 µl 10x Buffer RT, 100 µl dNTP Mix (contains 5 mM each dNTP), 1.1 ml RNase-free water	205211
HotStarTaq Master Mix Kit (250 U)	3 x 0.85 ml HotStarTaq Master Mix (contains 250 units HotStarTaq DNA Polymerase, PCR Buffer with 3 mM $MgCl_2$, and 400 µM of each dNTP) and 2 x 1.7 ml RNase-free water	203443

* The Sensiscript RT Kit (50) is sufficient for only 25 samples using AdnaTest ProstateCancerDetect because twice the volume is required for each reaction.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Changes
03/2017	Initial release
04/2020	Increased volume of Oligo(dT)$_{25}$ Beads in the AdnaTest ProstateCancerDetect to 355 µl, from the previous 280 µl. Replaced handbooks with quick-start protocols in kit contents. Removed statement that license from Hoffmann-La Roche AG, Basel, is required to use AdnaTest ProstateCancerDetect, because that patent has expired. In "Analysis of the results for AdnaTest AR-Detect", added statement that a fragment larger than 900 bp indicates contamination with genomic DNA. In "Sample preparation", changed blood storage temperature to 2–8°C, from 4–8°C.

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