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AdnaTest ProstateCancerSelect and ProstateCancerDetect Handbook

Σ 12 (catalog number 395432)

Σ 12 (catalog number 396432)

For enrichment of tumor cells from the whole blood of prostate cancer patients and detection of prostate cancer associated gene expression in enriched tumor cells
For in vitro diagnostic use
Version 1

IVD

CE

REF 395432 (AdnaTest ProstateCancerSelect)
396432(AdnaTest ProstateCancerDetect)

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Sample to Insight

QIAGEN

Contents

Intended Use

The AdnaTest ProstateCancerSelect is an in vitro diagnostic method intended for the immunochemical enrichment of circulating tumor cells from anti-coagulated whole blood samples obtained from prostate cancer patients, through a combination of epithelial and tumor associated antigens.

The AdnaTest ProstateCancerDetect is an in vitro diagnostic assay intended for the analysis of expression profiles of tumor cells by reverse transcription and multiplex PCR and subsequent densitometric analysis of the PCR products by automated capillary electrophoresis utilizing the Agilent® 2100 Bioanalyzer.

The AdnaTest ProstateCancerSelect/Detect is not intended for screening purposes and not to be used as a diagnostic test to confirm the presence of prostate cancer.

The product is intended to be used by professional users, such as technicians and physicians who are trained in molecular biological techniques.

Summary and Explanation

AdnaTest ProstateCancerSelect enables the immunomagnetic enrichment of tumor cells via epithelial and tumor associated antigens. AdnaTest ProstateCancerDetect is used for analysis of prostate cancer associated gene expression in immunomagnetically enriched tumor cells by reverse transcription and PCR.

Principle of the Procedure

AdnaTest ProstateCancerSelect

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).

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

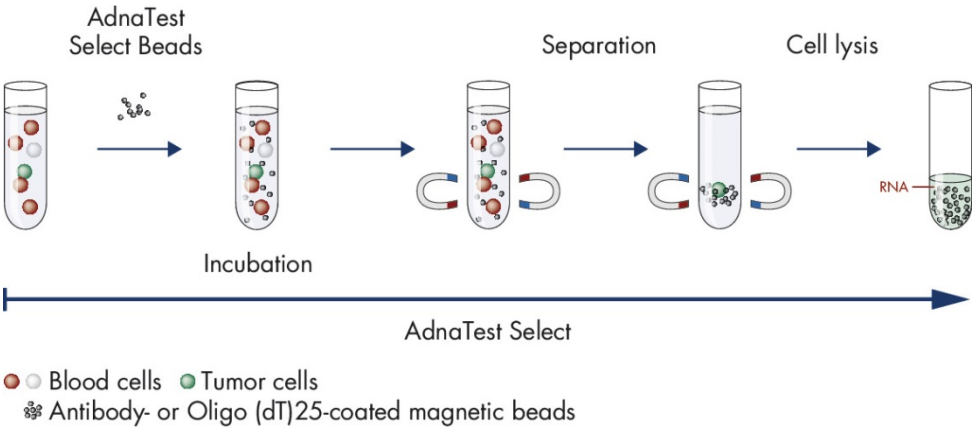

Figure 1. AdnaTest ProstateCancerSelect: Immunomagnetic cell selection with multiple tumor associated antibodies. In the first step, the CTCs in the blood are enriched (AdnaTest Select). This is achieved using antibody-coated magnetic particles (beads). Several antibodies are used, which bind with high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

AdnaTest ProstateCancerDetect

AdnaTest ProstateCancerDetect contains Oligo $(dT)_{25}$ Beads for the isolation of mRNA from the lysate of pre-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 three tumor associated antigens and one control gene. The AdnaTest PrimerMix AR-Detect allows amplification of androgen receptor (AR).

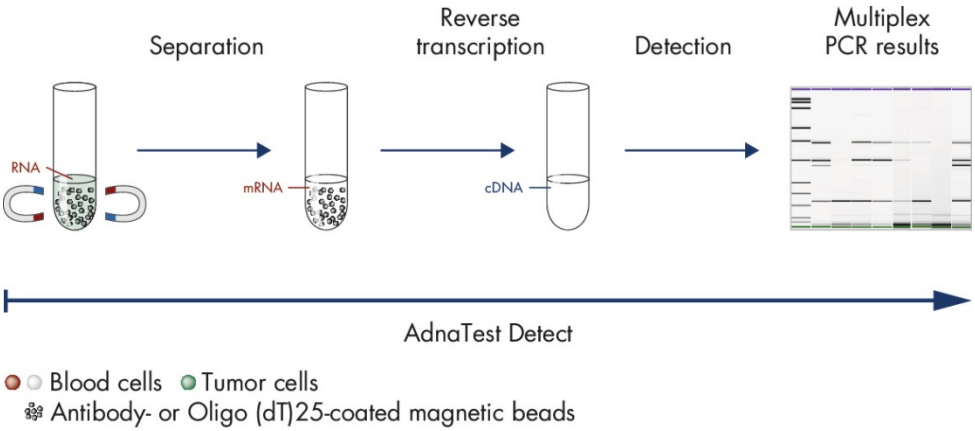

Figure 2. AdnaTest ProstateCancerDetect: Multiplex PCR of various cancer associated tumor markers. In a second step the enriched cells are examined by RT-PCR for tumor associated expression patterns. The mRNA strands are reverse transcribed into cDNA. Subsequently, several associated tumor markers can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

The two AdnaTest PrimerMixes generate the following fragments:

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.

Materials Provided

Kit contents

AdnaTest ProstateCancerSelect			
Number of tests			**12**
Catalog number			**395432**
Collection Tubes	Collection Tubes (15 ml)		3 x 5
Collection Tubes	Collection Tubes (1.5 ml)		24
Red	ProstateSelect Beads	PSB	1.2 ml
Red	AdnaTest Lysis/Binding Buffer	LBB	2 x 1.2 ml
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AdnaTest ProstateCancerDetect			
Catalog no.			**396432**
Number of tests			**12**
AdnaTest RNA Reagents			**Box 1**
Red	AdnaTest Lysis/Binding Buffer	LBB	2 ml
Orange	Oligo(dT)25 Beads	OdT	280 µl
White	RNA Purification Buffer A	BA	4ml
White	RNA Purification Buffer B	BB	4ml
Purple	Tris-HCL Buffer	TB	2 ml
AdnaTest ProstateCancerDetect			**Box 2**
Blue	AdnaTest PrimerMix ProstateDetect	PMP	144 µl
Orange	AdnaTest Positive Control Prostate (C+)	CONTROL +	40 µl
Yellow	AdnaTest PrimerMix AR-Detect	PMA	144 µl
Pink	AdnaTest Positive Control AR (C+)	CONTROL +	40 µl
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The AdnaTest ProstateCancerDetect reagents are sufficient to analyze 6 PCR controls and 12 blood samples.

Materials Required but Not Provided

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
- 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 (e.g., ELMI Ltd., cat. no. IMIX-03)
- Magnetic particle concentrator AdnaMag-S (cat. no. 399911)
- Thermal block or water bath (65°C)
- Thermal cycler with a heated lid and a heating rate of 2°C/s.
- Agilent 2100 Bioanalyzer (Agilent Technologies)

Material

- Sterile, RNase-free thin-wall 0.2 ml PCR tubes
- 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 1 µl to 200 µl

Reagents

- Sensiscript® RT Kit (QIAGEN, cat. no. 205211, 50 reactions)
  - **Note**: The Sensiscript RT Kit (cat. no. 205211) 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 (QIAGEN, cat. no. 203443, 250 U)
- Crushed ice

Warnings and Precautions

For in vitro diagnostic use

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.

Discard sample and assay waste according to your local safety regulations.

Application information

These tests must be performed by personnel skilled in molecular biological techniques.

Patents

AdnaTest ProstateCancerDetect requires licenses of Hoffmann-La Roche AG, Basel. The purchase of AdnaTest ProstateCancerDetect does not authorize the user to perform the PCR without license.

Reagent Storage and Handling

Storage

The AdnaTest ProstateCancer system is delivered in three boxes. AdnaTest ProstateCancerSelect (cat. no. 395432) and the AdnaTest RNA Reagent Box 1 (Box 1 of cat. no. 396432) 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. 396432), containing the AdnaTest PrimerMixes and AdnaTest Positive Controls, must be stored separately 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.

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. Observe the safety information 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).

Specimen Handling and Storage

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 (Becton Dickinson GmbH, cat. no. 366645 [EU]; 364606 [US]) 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 Sample Tube, cat. no. 399932.

- Blood must be stored at 4–8°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.

Protocol: Enrichment of Tumor Cells Using AdnaTest ProstateCancerSelect

Important points before starting

- Before beginning the procedure, read "Warnings and Precautions" (page 10), "Reagent Storage and Handling" (page 11) and "Specimen Handling and Storage" (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".
- Please 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 a 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; 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 processed use additional 1.5 ml reaction tubes (not provided).
3. Place the tube into the AdnaMag-S.
4. After 1 minute remove the supernatant with a pipette.

   Note: Do not touch the beads when removing the supernatant!
5. Wash steps:

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

5b. Add 1 ml PBS and resuspend the beads by repeated pipetting.
5c. Place the magnet slider into the AdnaMag-S.
5d. After 1 minute remove the supernatant completely with a pipette.
5e. Repeat steps 5a to 5d twice (three washes in total).

6. Remove the tube from the AdnaMag-S, and resuspend the beads in PBS to the original volume (100 µl per sample). Proceed with "Procedure B: Selection of tumor cells", below.

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 minutes at room temperature on a device allowing both tilting and rotation.
4. Place tubes into the AdnaMag-L without the magnet slider. Swing the AdnaMag-L downwards to release blood drops captured in the cap.
5. Insert the magnet slider and incubate the tubes in the AdnaMag-L for 3 minutes at room temperature.
6. Remove blood supernatant completely with a 10 ml pipette without touching the beads.

   Note: Do not touch the beads when removing the supernatant!
7. Wash steps:
   7a. Remove the magnet slider from the AdnaMag-L.
   7b. Add 5 ml PBS. Close the tubes and shake the AdnaMag-L gently back and forth 5 times to resuspend the magnetic bead/cell complexes.
   7c. Swing the AdnaMag-L with the tubes downwards twice to release drops captured in the cap.

7d. Place the magnet slider into the AdnaMag-L and incubate for 1 minute at room temperature.

7e. Remove supernatant completely with a pipette.

7f. Repeat steps 7a to 7e twice (three washes in total).

8. Remove the magnet slider from the AdnaMag-L.

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 with an inserted magnet slider.

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

11. After 1 minute remove the supernatant completely with a pipette to optimize the following cell lysis.

12. Remove the magnet slider from the AdnaMag-S.

13. Add 200 µl AdnaTest Lysis/Binding Buffer (equilibrated to room temperature) to each reaction tube. Resuspend by pipetting at least five times.

14. Insert the magnet slider into the AdnaMag-S, and incubate for 1 minute.

15. Transfer supernatant (cell lysate) into new 1.5 ml reaction tubes (provided).

16. Discard the tubes with 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 –20°C for a maximum of 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 "Warnings and Precautions" (page 10) and "Reagent Storage and Handling" (page 11).
- Procedures A to C describe the isolation of mRNA and reverse transcription.
- Please 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 a 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; 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.

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

4. After 1 minute remove the supernatant with a pipette.
5. Wash steps:
   5a. Remove the magnet slider from the AdnaMag-S.
   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.
   5d. After 1 minute remove the supernatant completely.
   5e. Repeat steps 5a to 5d once (two washes in total).
6. Remove the tube from the AdnaMag-S, 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 minutes at room temperature on a device allowing both tilting and rotation.
3. Place the tubes into the AdnaMag-S without the magnet slider. Swing the AdnaMag-S downwards to release beads and liquid captured in the cap.
4. Insert the magnet slider and remove the supernatant after 1 minute.
5. Wash steps 1:
   5a. Remove the magnet slider from the AdnaMag-S.
   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.

5d. After 1 minute remove the supernatant completely.

5e. Repeat steps 5a to 5d once (two washes in total).

6. Wash steps 2:

   6a. Remove the magnet slider from the AdnaMag-S.

   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.

   6d. After 1 minute remove the supernatant completely. This step must be carried out carefully (watch the pellet) since the beads may slide and could be removed by mistake.

   6e. Repeat steps 6a to 6d once in the same reaction tubes (two washes in total).

7. Remove the magnet slider from the AdnaMag-S.
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.
10. After 1 minute remove the supernatant completely.
11. Remove the magnet slider from the AdnaMag-S.
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 minutes at 65°C.
14. Place the tubes on ice immediately for at least 2 minutes.
15. Continue immediately (within 5 minutes) with reverse transcription (Procedure C: Reverse transcription using the Sensiscript RT Kit).

    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 calculated for the total number of reverse transcription reactions. A negative control reaction without addition of mRNA must always be prepared (RT control).

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.

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!

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

Table 2. Reverse transcription program

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

5. Place reaction tubes with the cDNA on ice or store at –20 °C for a maximum of 4 weeks.

Continue with "Protocol: Multiplex and Singleplex PCR", page 21.

Protocol: Multiplex and Singleplex PCR

Important point before starting

- Before beginning the procedure, read “Warnings and Precautions” (page 10) and “Reagent Storage and Handling” (page 11).

Things to do before starting

- Thaw HotStarTaq Master Mix (QIAGEN), 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. The PCR master mix is prepared 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.

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: As negative control add 4.0 µl of RNase-free water instead of cDNA.

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**

3. A thermal cycler is used 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

	Temperature	Time
Initial activation step	95°C	15 minutes
3-step cycling Denaturation:	94°C	30 seconds
Annealing:	61°C	30 seconds
Extension:	72°C	30 seconds
Number of cycles:	42	
Final extension:	72°C	10 minutes
Cool-down:	4°C	∞

Procedure B: Singleplex PCR (AdnaTest AR-Detect)

1. The PCR master mix is prepared 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.

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: As 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**

3. A thermal cycler is used 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

	Temperature	Time
Initial activation step	95°C	15 minutes
3-step cycling (35 cycles) Denaturation:	94°C	30 seconds
Annealing:	60°C	30 seconds
Extension:	72°C	60 seconds
Number of cycles:	35	
Final extension:	72°C	10 minutes
Cool-down:	4°C	∞

Interpretation of Results

Fragment analysis on the Agilent 2100 Bioanalyzer

Analysis is performed with the Agilent 2100 Bioanalyzer (Agilent Technologies) 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. Select **Data** under **Contexts** 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 one tumor associated transcript (PSMA, PSA or EGFR) is clearly detected.

Using the Agilent 2100 Bioanalyzer, peaks with a concentration of ≥ 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.

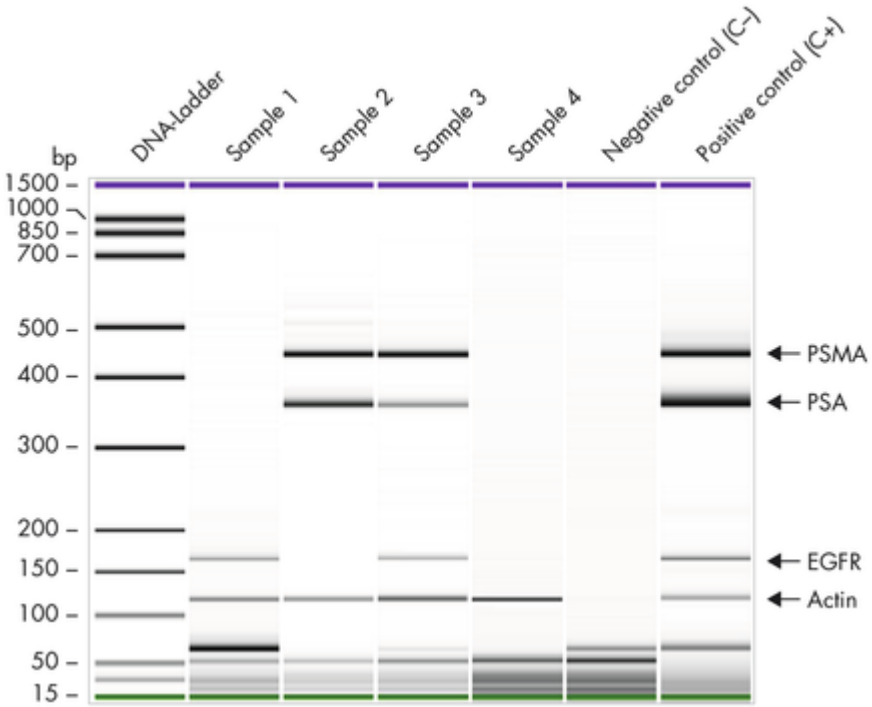

Figure 3. AdnaTest ProstateCancerDetect results of multiplex PCR samples analyzed with an Agilent 2100 Bioanalyzer. The first lane shows the DNA size standard (DNA-Ladder). Sample 1 is positive for EGFR, sample 2 is positive for PSMA and PSA, and sample 3 is positive for PSMA, PSA and EGFR. Sample 4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

Analysis of the results for AdnaTest AR-Detect

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

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 singleplex PCR. The negative control and the RT control samples must not show any bands larger than 80 base pairs (primer-dimers).

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

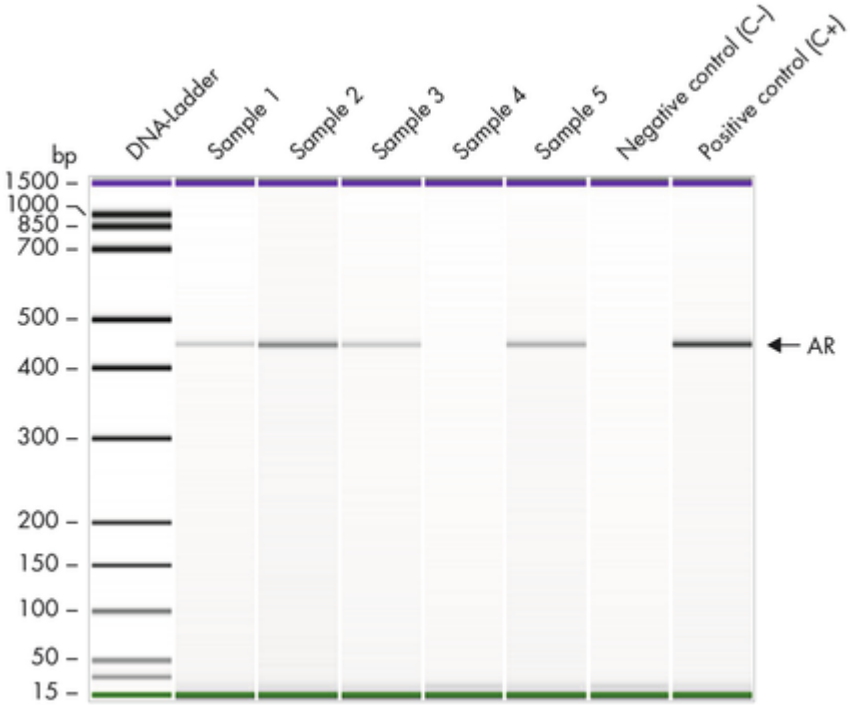

Figure 4. AdnaTest ProstateCancerDetect results of singleplex PCR samples. The first lane shows the DNA size standard (DNA-Ladder). Samples 1 to 3 and sample 5 are positive for AR. Sample 4 is negative. The PCR negative (C-) and positive control (C+) are shown in the last two lanes.

Troubleshooting guide

This troubleshooting guide may be helpful in solving any problems that may arise. 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**).

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.

Limitations

All reagents may exclusively be used in in vitro diagnostics.

The product is only to be used by personnel specially instructed and trained in in vitro diagnostics procedures.

It is important that the operator reads the instructions for use thoroughly before using the system.

Strict compliance with the instructions for use is required for optimal PCR results.

Check the expiration dates printed on the box and labels of all components. Do not use components beyond their expiration date.

Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

Performance Characteristics

Recovery

Two cultured LnCap prostate cancer cells were spiked into blood samples from healthy donors to determine the recovery rates achieved with AdnaTest ProstateCancerSelect/Detect (Table 7).

Table 7. AdnaTest ProstateCancer recovery rate of tumor cells spiked into blood samples from healthy donors

	Number of positives	Total number of samples
Two tumor cells spiked into 5 ml blood	38 (95%)	40

The recovery rate is 95% for detection of 2 tumor cells spiked into 5 ml of blood from healthy donors.

Specificity

AdnaTest ProstateCancerSelect/Detect was used to analyze 40 healthy donors to determine the rate of false positives at the given cut-off (0.10 ng/µl fragment concentration for each gene profile included, except for actin).

Table 8. Determination of specificity

Controls	Total number of samples	Number of false positives	Specificity (%)
Healthy donors	40	0 (0%)	100

AdnaTest ProstateCancerSelect/Detect demonstrated a specificity of 100% (Table 8).

Reproducibility

Twenty blood samples from healthy donors were spiked with 10 LnCap prostate cancer cells per sample. Blood samples were analyzed by two operators using AdnaTest ProstateCancerSelect/Detect to determine the reproducibility. The intra-assay and the inter-assay reproducibility were 100% (Table 9).

Table 9. Reproducibility of AdnaTest ProstateCancer Select/Detect

Operator	Positive AdnaTest result/samples	Intra-assay reproducibility (%)	Inter-assay reproducibility (%)
A	10/10	100	100
B	10/10	100	100

Precision

To determine the precision, aliquots of cDNA were pooled and analyzed using AdnaTest ProstateCancerDetect. Two operators analyzed 30 cDNA samples, consisting of 3 independent measurements of 10 samples. The intra-assay and inter-assay precision were 100% (Table 10).

Table 10. Precision of AdnaTest ProstateCancerDetect

Operator	Positive AdnaTest result/samples	Intra-assay precision (%)	Inter-assay precision (%)
A	30/30	100	100
B	30/30	100	100

Interfering substances

Anticoagulants

When drawing and transporting blood, use of anticoagulants is mandatory. However, heparin and citrate lead to aggregate formation after addition of AdnaTest immunomagnetic beads,

which can result in a lack of test results or false test results. However, EDTA and ACDA (citrate/dextrose/adenine solution A) are compatible with AdnaTest immunomagnetic beads.

Hemolysis

Hemolysis in blood samples (plasma fraction appears red) is, in most cases, due to incorrect transportation or storage conditions. Such samples may give false-negative results and should be discarded.

Chemotherapeutics, targeted therapy drugs and anti-hormonal regimens

Chemotherapeutics (taxanes, cisplatin, oxaliplatin, 5-FU, anthracycline, irinotecan etc.) are potent cytotoxins and cause damage or rapid cell death in a blood sample. This results in a high likelihood of false-negative results when using AdnaTest immunomagnetic beads. After administration of these substances, the human body needs around 5–7 days to detoxify them (Table 11). Blood samples drawn during this time must not be used with AdnaTest immunomagnetic beads.

Table 11. Half-lives of chemotherapeutics

Drug	Half life	Reference
5-Fluouracil	Up to 20 minutes	**www.drugs.com/pro/fluorouracil-injection.html**
Docetaxel	Up to 11.1 hours	**www.drugs.com/pro/docetaxel.html**
Cis-platinum	Up to 30 minutes	**www.drugs.com/pro/cisplatin.html**
Carbo-platinum	Up to 5.9 hours	**www.drugs.com/pro/carboplatin.html**
Paclitaxel	Around 25.4 hours	**www.drugs.com/pro/paclitaxel.html**

The same precaution is also recommended for targeted therapy regimens such as antibodies (Herceptin®, bevacizumab, cetuximab etc.), tyrosine kinase blockers (olaparib, Iressa®, Erbitux®, lapatinib etc.) and anti-hormonal drugs (tamoxifen, abiraterone, enzalutamide etc.) administered as a single drug or in combination with chemotherapeutics.

In clinical trials demonstrating the prognostic value of circulating tumor cells (CTC) identified and characterized using AdnaTest immunomagnetic beads, no negative interference of

chemotherapeutics, targeted therapies or anti-hormonal therapies was observed, provided the waiting period of at least 7 days after administration of the drug was complied with. Furthermore, a negative impact of common co-medications (Aspirin, ibuprofen, aprepitant, steroids etc.) is unlikely but is being monitored.

Interfering conditions

Blood clotting

In the context of clinical trials, we observed blood clotting after incubation with *AdnaTest* immunomagnetic beads – most frequently in blood samples from patients in a late disease state. Blood samples that exhibit clotting are difficult to process during the *AdnaTest* workflow due to increased viscosity and are difficult to pipet. They also contain an unacceptably high number of contaminating leukocytes, which leads to false-positive results. Such samples must be discarded.

Benign organic disease and chronic inflammatory conditions

Benign organic disease and chronic inflammation, such as arthritis, benign prostatic hyperplasia (BPH), Crohn's disease etc., do not lead to false-positive AdnaTest results.

Acute allergy

With acute allergic conditions, there is an increased number of contaminating leucocytes after CTC enrichment using AdnaTest immunomagnetic beads. Therefore, false-positive results cannot be fully excluded.

Clinical studies

A total of 12 patients with metastatic castrate-resistant prostate cancer (CRPC) were followed during docetaxel treatment. A first sample was analyzed at baseline and 2 further samples were analyzed during follow-up.

With regards to AR activation, it was clearly demonstrated that AR activation and deactivation correlated strongly with the rate of CTC elimination due to therapeutic intervention. However, the CTC positivity rate dropped during the course of therapy from 70% at baseline to ~35% during follow-up and AR positivity decreased from 55% to ~11%. Due to the therapy, AR-positive CTC subclones are more affected by docetaxel treatment than AR-negative CTC. These findings correlate well with those from Darshan et al. 2011, in which a taxane-induced blockade of AR nuclear transport and signaling was observed.

These findings indicate the specific and sensitive detection of CTCs in prostate cancer clinical samples as well as an assessment of genetic profiles related to therapeutic targets.

Reference

Darshan, M.S. et al. (2011) Taxane-Induced Blockade to Nuclear Accumulation of the Androgen Receptor Predicts Clinical Responses in Metastatic Prostate Cancer. Cancer Res. 2011 Sep 15; **71(18)**: 6019–6029. Published online 2011 Jul 28. doi: 10.1158/0008-5472.CAN-11-1417.

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

 <N>	Contains reagents sufficient for <N> tests
	Use by
	Temperature limitation
	Catalog number
	Consult instructions for use
	Manufacturer
	In vitro diagnostic medical device
	Material number
	Global Trade Item Number

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	395432
AdnaTest ProstateCancerDetect	RT-PCR kit for detection of prostate cancer-associated gene expression in enriched tumor cells	396432
Related products		
AdnaTubes	12 sample tubes containing EDTA. Use only with anticoagulated blood collected in A-CDA blood collection tubes from BD	399932
AdnaMag-L	For 8 tubes, 15 ml	399921
AdnaMag-S	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.

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