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AdnaTest OvarianCancerSelect and OvarianCancerDetect Handbook

For enrichment of tumor cells from whole
blood in ovarian cancer research and
detection of cancer associated gene
expression in enriched tumor cells
For molecular biology applications

REF

395042 (AdnaTest OvarianCancerSelect)

396042 (AdnaTest OvarianCancerDetect)



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Kit Contents

AdnaTest OvarianCancerSelect	
Catalog no.	395042
Number of tests	12
Collection Tubes (1.5 ml)	3 x 5
Collection Tubes (15 ml)	24
OvarianSelect Beads	1.2 ml
AdnaTest Lysis/Binding Buffer	2 x 1.2 ml
Handbook	1

AdnaTest OvarianCancerDetect	
Catalog no.	396042
Number of tests	12
AdnaTest RNA Reagent	
Box 1	
AdnaTest Lysis/Binding Buffer	2 ml
Oligo(dT) ₂₅ Beads	280 µl
RNA Purification Buffer A	4 ml
RNA Purification Buffer B	4 ml
Tris-HCL Buffer	2 ml
AdnaTest OvarianCancerDetect	
Box 2	
AdnaTest PrimerMix OvarianDetect	144 µl
AdnaTest Positive Control Ovarian (C+)	56 µl
AdnaTest PrimerMix ERCC1-Detect	144 µl
AdnaTest Positive Control ERCC1 (C+)	56 µl
Handbook	1

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

Storage

The AdnaTest OvarianCancer system is delivered in three boxes. AdnaTest OvarianCancerSelect (cat. no. 395042) and the AdnaTest RNA Reagent Box 1 (Box 1 of cat. no. 396042) have to be stored at 2–8°C. The components must not be used beyond the expiration date.

AdnaTest OvarianCancerDetect Box 2 (Box 2 of cat. no. 396042), containing the AdnaTest PrimerMixes and AdnaTest Positive Controls, must be stored separately at –30 to –15°C. In order 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 OvarianCancerSelect and AdnaTest OvarianCancerDetect 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)

bp	Base pairs
C+	Positive control
C-	Negative control
CA125	Cancer antigen 125
cDNA	Complementary deoxyribonucleic acid
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
ERCC1	Excision repair cross-complementing 1
GA733-2	Gastrointestinal tumor associated antigen 733-2
kb	kilobases
mRNA	Messenger ribonucleic acid
Muc-1	Muc-1 gene
PCR	Polymerase chain reaction
RNase	Ribonuclease
rpm	Revolutions per minute
RT	Reverse transcription

Symbols



Use by



Temperature limitation



Catalog number



Consult instructions for use



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 OvarianCancerSelect and AdnaTest OvarianCancerDetect is tested against predetermined specifications to ensure consistent product quality.

Introduction

The AdnaTest OvarianCancer system is used for the enrichment and molecular characterization of circulating tumor cells (CTCs) from whole blood in ovarian cancer research. Subsequently, the AdnaTest OvarianCancerDetect is used for analysis of ovarian 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% and 2 tumor cells in 5 ml of whole blood can be detected at a recovery rate of at least 70%.

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. 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 two-step process (Select and Detect) to generate results within 5 hours.

AdnaTest OvarianCancerSelect

AdnaTest OvarianCancerSelect 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 (Figures 1 and 2).

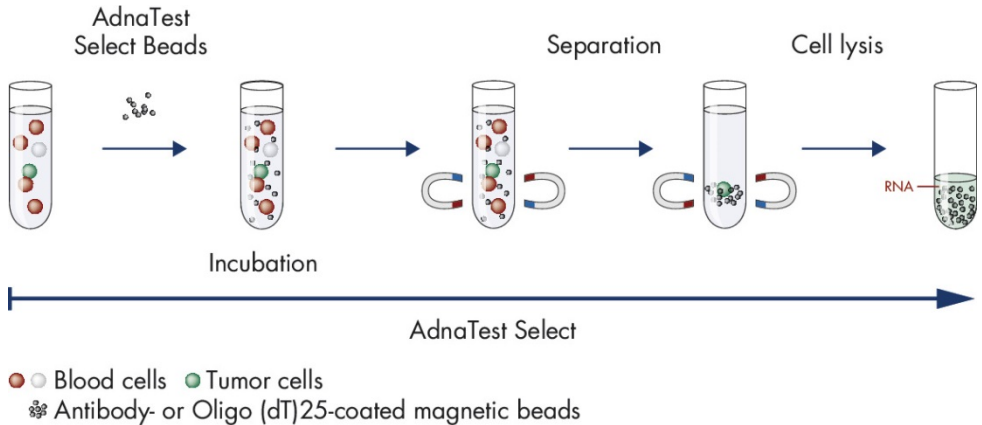
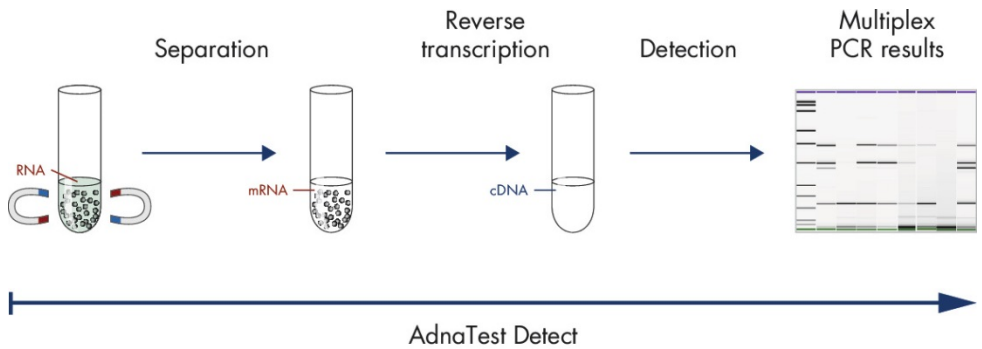


Figure 1. AdnaTest OvarianCancerSelect: Immunomagnetic cell selection with multiple tumor associated antibodies.

The cell lysate is used for further analysis (it is recommended to continue with AdnaTest OvarianCancerDetect).

AdnaTest OvarianCancerDetect

AdnaTest OvarianCancerDetect 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/duplex-PCR. The AdnaTest PrimerMix OvarianDetect allows the amplification of three tumor associated antigens and one control gene. The AdnaTest PrimerMix ERCC1-Detect amplifies the excision repair cross-complementing 1 gene (ERCC1) and one control gene.



- Blood cells ● Tumor cells
- ⊗ Antibody- or Oligo (dT)25-coated magnetic beads

Figure 2. AdnaTest OvarianCancerDetect: 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:

AdnaTest PrimerMix OvarianDetect

- CA125 : 432 bp
- GA733-2: 395 bp
- Muc-1: 299 bp
- Actin: 120 bp (internal PCR control)

AdnaTest PrimerMix ERCC1-Detect

- ERCC1: 357 bp
- Actin: 120 bp (internal PCR control)

Note: Fragment sizes may vary slightly. Please use the AdnaTest Positive Control Ovarian and AdnaTest Positive Control ERCC1 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 OvarianCancerSelect

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

- AdnaTubes (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 OvarianCancerDetect

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 (50°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 (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, 2.500 U (Promega, cat. no. N2511)
- HotStarTaq® Master Mix Kit (QIAGEN, cat. no. 203443, 250 U)
- Crushed ice

Important Notes

Application information

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


Patents

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

Sample preparation

- Blood samples must be taken before the application of therapeutic substances. Do not use the AdnaTest OvarianCancerSelect 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, cat. no. 399932.
- Blood must be stored at 4°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

- OvarianSelect 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 OvarianCancerSelect”, page 14.)
- All components and additional reagents provided by other suppliers must be stored according to their instructions. Safety advice of the respective manufacturers is valid.
- Wear protective gloves to avoid contamination with DNA, RNA and RNases.
- Aliquot the OvarianSelect 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 other suppliers than suggested may adversely affect the results.
- The safety and hygiene regulations of the laboratory must be respected (e.g., wear lab coats, protective goggles, gloves).

Protocol: Enrichment of Tumor Cells Using AdnaTest OvarianCancerSelect

Important points before starting

- Before beginning the procedure, read “Important Notes” (page 12).
- It is necessary to remove sodium azide by washing the OvarianSelect Beads prior to use, as described below in “Procedure A: Preparation of the OvarianSelect Beads”.

Things to do before starting

- Ensure that AdnaTest Lysis/Binding Buffer is equilibrated to room temperature. If a precipitate is observed, equilibrate the reagents to room temperature and mix until the precipitate is completely dissolved.

Procedure A: Preparation of the OvarianSelect Beads

1. Resuspend the OvarianSelect Beads thoroughly by pipetting; do not vortex!
2. Calculate the volume of OvarianSelect Beads required for all samples to be processed (100 μ l 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.

3. Place the tube into the AdnaMag-S.
4. After 1 min 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 min 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 μ l 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 (provided).

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 OvarianSelect Beads thoroughly (prepared in step 6 of Procedure A) by pipetting, and add 100 μ l of these beads to each blood sample.
3. Rotate tubes slowly (approximately 5 rpm) for 30 min 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 min 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 min 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.
 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 min 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 μ l 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 min.
 15. Transfer each supernatant (cell lysate) into a new 1.5 ml reaction tube (provided).
 16. Discard the tubes with the beads.
 17. Continue with mRNA isolation (see "Protocol: Detection of Ovarian Cancer Associated Gene Expression in Enriched Tumor Cells Using AdnaTest OvarianCancerDetect", page 17) immediately, or store the cell lysates at -20°C for no longer than 2 weeks.

Protocol: Detection of Ovarian Cancer Associated Gene Expression in Enriched Tumor Cells Using AdnaTest OvarianCancerDetect

Important points before starting

- Before beginning the procedure, read “Important Notes” (page 12).
- Procedures A to C describe the isolation of mRNA and reverse transcription.

Things to do before starting

- Ensure that AdnaTest Lysis/Binding Buffer is equilibrated to room temperature. If a precipitate is observed, equilibrate the reagents 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 50°C.

Procedure A: Preparation of Oligo(dT)₂₅ Beads

1. Resuspend the Oligo(dT)₂₅ Beads thoroughly by pipetting. 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 min 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 min 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 μ l 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 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 min.
5. Wash steps 1:
 - 5a. Remove the magnet slider from the AdnaMag-S.
 - 5b. Add 100 μ l 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 min 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 μ l 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 min remove the supernatant completely. This step has to be carried out carefully (watch the pellet) since the beads might 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 μ l 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 min remove the supernatant completely.
11. Remove the magnet slider from the AdnaMag-S.
12. Resuspend the mRNA/bead-complex in 29.5 μ l RNase-free water.
13. Transfer the tubes to a thermal block or water bath, and incubate for 5 min at 50°C.
14. Place the tubes on ice immediately for at least 2 min.
15. Continue immediately (within 5 min) with the reverse transcription (Procedure C).

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 10.5 μ l for each reaction into 0.2 ml PCR tubes.
3. Resuspend the mRNA/bead complexes (step 10, 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	4.0 μ l
dNTP Mix (5 mM each dNTP)	4.0 μ l
RNase inhibitor, 40 U/ μ l (Promega)	0.5 μ l
Sensiscript Reverse Transcriptase	2.0 μ l
Template RNA*	29.5 μ l
mRNA/bead complex or RNase free water	
Total volume	40.0 μl

* As RT control add 29.5 μ l 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.

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

Table 2. Reverse transcription program

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

5. Place reaction tubes with the cDNA on ice or store at -20°C for a maximum of 4 weeks.
6. Continue with "Protocol: Multiplex and Duplex PCR and Fragment Analysis", page 21.

Protocol: Multiplex and Duplex 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 (QIAGEN), AdnaTest PrimerMix OvarianDetect, AdnaTest PrimerMix ERCC1-Detect, AdnaTest Positive Control Ovarian, AdnaTest Positive Control ERCC1 and RNase-free water. Vortex, centrifuge quickly and store on ice.

Procedure A: Multiplex PCR (AdnaTest OvarianDetect)

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

The volume of the PCR Master Mix should be at least 10% greater than the requirement calculated from the number of samples. Note that an AdnaTest Positive Control Ovarian, RNase-free water as negative control and the RT Control must always be included.

2. For each preparation dispense 42.0 µl of the Master Mix into 0.2 ml PCR reaction tubes. Resuspend the cDNA/bead mix by pipetting, and add 8.0 µl of it to each reaction tube.

Note: As negative control add 8.0 µl of RNase-free water instead of cDNA.

Table 3. Preparation of the multiplex PCR

Component	Volume
Multiplex PCR master mix	
HotStarTaq Master Mix	25.0 µl
RNase-free water	13.0 µl
AdnaTest PrimerMix OvarianDetect	4.0 µl
cDNA or RT control or Negative control (RNase-free water) or AdnaTest Positive Control Ovarian, each:	8.0 µl
Total volume	50.0 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 37 cycles.

Table 4. PCR cycling program

Step	Time	Temperature
Initial activation step	15 min	95°C
3-step cycling		
Denaturation	30 s	94°C
Annealing	30 s	58°C
Extension	30 s	72°C
Final extension	10 min	72°C
Cooling	∞	12°C

Procedure B: Duplex PCR (AdnaTest ERCC1-Detect)

1. The PCR Master Mix is prepared as shown in Table 5 according to the number of samples.

The volume of the Master Mix should be at least 10% larger than the requirement calculated from the number of samples. Note that an AdnaTest Positive Control ERCC1, RNase-free water as negative control and the RT control must always be included.

2. For each preparation dispense 42.0 μl of the Master Mix into 0.2 ml PCR reaction tubes. Resuspend the cDNA/bead mix by pipetting, and add 8.0 μl of it to each reaction tube.

Note: As negative control add 8.0 μl of RNase-free water instead of cDNA.

Table 5. Preparation of the duplex PCR

Component	Volume
Duplex PCR master mix	
HotStarTaq Master Mix	25.0 μl
RNase-free water	13.0 μl
AdnaTest PrimerMix ERCC1-Detect	4.0 μl
cDNA or RT control or Negative control (RNase-free water) or AdnaTest Positive Control ERCC1, each:	8.0 μl
Total volume	50.0 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

Step	Time	Temperature
Initial activation step	15 min	95°C
3-step cycling		
Denaturation	30 s	94°C
Annealing	30 s	60°C
Extension	60 s	72°C
Final extension	10 min	72°C
Cooling	∞	12°C

Procedure C: Fragment analysis on the Agilent 2100 Bioanalyzer

We recommend performing the analysis 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 can cause false 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 OvarianDetect

The test is considered positive, if a PCR fragment of at least one tumor associated transcript (GA733-2, Muc-1 or CA125) is clearly detected.

If you are using the Agilent 2100 Bioanalyzer, peaks with a concentration of ≥ 0.15 ng/ μ l are positive (Figure 3).

The fragment of the control gene actin must show in all test samples (internal PCR control). An actin signal provides a positive control for a 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 1000 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.

In case assistance is needed to interpret the results, please do not hesitate to contact our support team.

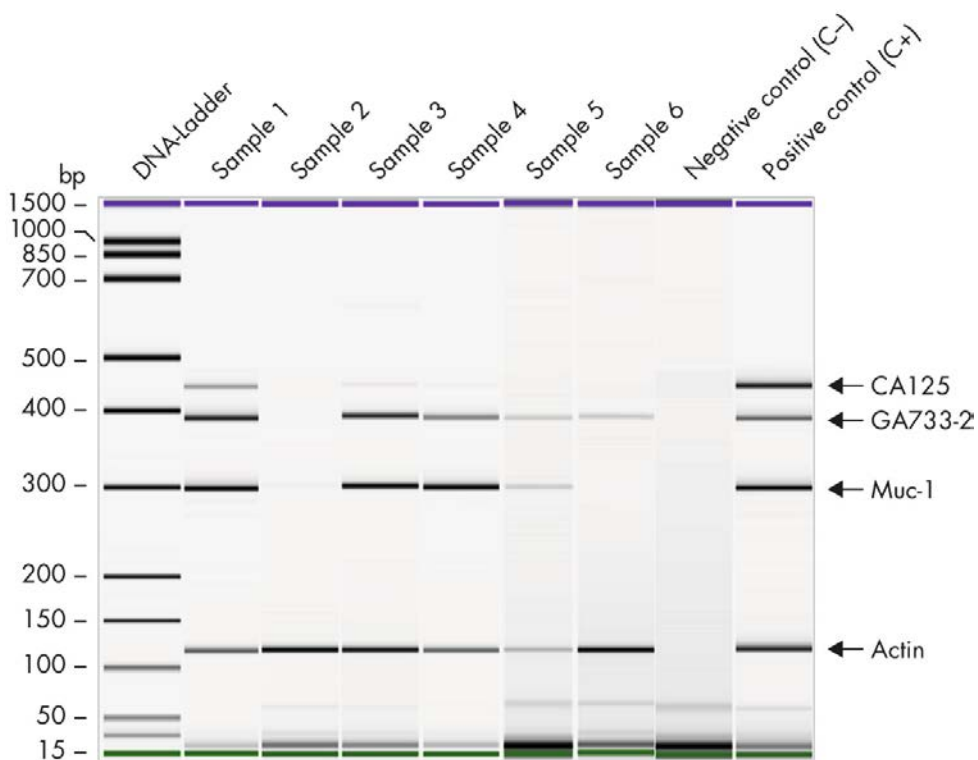


Figure 3. AdnaTest OvarianCancerDetect results of multiplex PCR samples analyzed with an Agilent 2100 Bioanalyzer. The first lane shows the DNA size standard (DNA-Ladder). Sample 1 is positive for GA733-2, Muc-1 and CA125; samples 3, 4 and 5 are positive for GA733-2 and Muc-1; and sample 6 is positive for GA733-2. Sample 2 is negative. Actin is detected in samples 1 to 6. The PCR negative control and positive control are shown in the last two lanes.

Analysis of the results for AdnaTest ERCC1-Detect

If you are using the Agilent 2100 Bioanalyzer, peaks with a concentration of ≥ 0.2 ng/ μ l for ERCC1 are positive (Figure 4).

The fragment of the control gene actin must show in all test samples (internal PCR control). An actin signal provides a positive control for a successful cell separation, reverse

transcription and duplex 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.

In case assistance is needed to interpret the results, please do not hesitate to contact our support team.

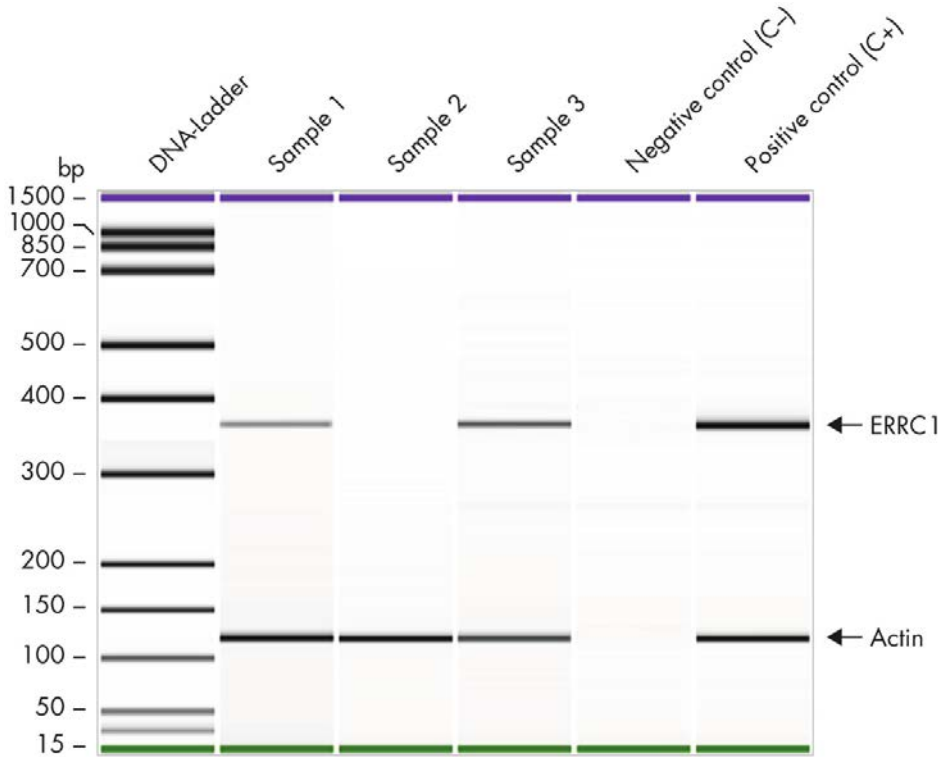


Figure 4. AdnaTest OvarianCancerDetect results of duplex PCR samples. The first lane shows the DNA size standard (DNA-Ladder). Samples 1 and 3 are positive for ERCC1. Sample 2 is negative. Actin is detected in samples 1 to 3. The PCR negative control and positive control (ERCC1) are shown in the last two lanes.

Troubleshooting Guide

See 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 OvarianCancerSelect	For isolation of CTCs and the subsequent extraction of mRNA from human whole blood for 12 preparations	395042
AdnaTest OvarianCancerDetect	RT-PCR kit for detection of ovarian cancer associated gene expression in enriched tumor cells	396042
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	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 OvarianCancerDetect* because twice the volume is required for each reaction.

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