April 2020

# 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)

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

Sample to Insight

QIAGEN

# Contents

# 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 |
| Quick-Start Protocol | 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 | 355 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 |
| Quick-Start Protocol | 1 |

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

# Shipping and Storage

The AdnaTest OvarianCancer system is delivered in 3 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 in a constant-temperature freezer 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: The AdnaTest OvarianCancerSelect is used for enriching CTCs from whole blood, while the AdnaTest OvarianCancerDetect is subsequently used for the 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%.

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 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 (Figure 1 and Figure 2).

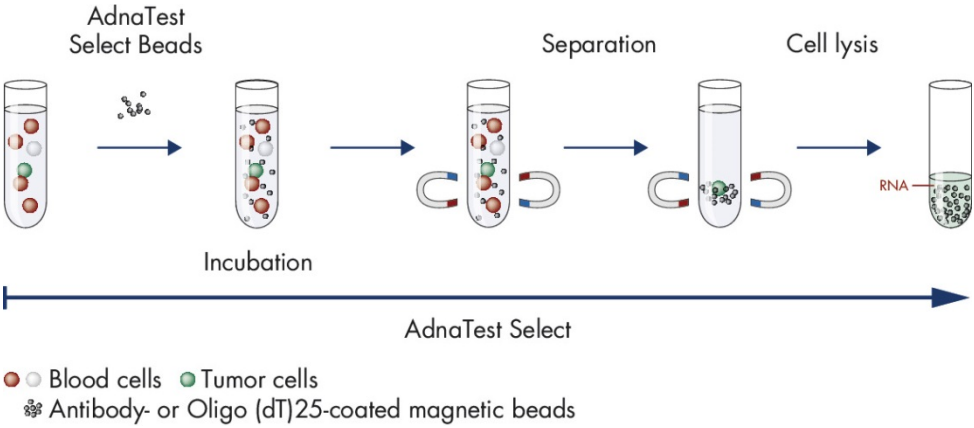


**Figure 1. AdnaTest OvarianCancerSelect: Immunomagnetic cell selection with multiple tumor associated antibodies.**

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

## AdnaTest OvarianCancerDetect

AdnaTest OvarianCancerDetect 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/duplex-PCR. The AdnaTest PrimerMix OvarianDetect allows the amplification of 3 tumor-associated antigens and 1 control gene. The AdnaTest PrimerMix ERCC1-Detect amplifies the excision repair cross-complementing 1 gene (ERCC1) and 1 control gene.

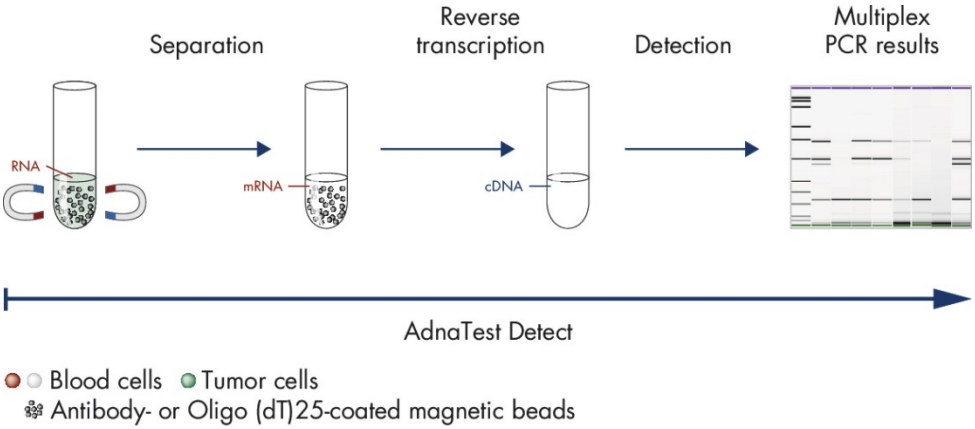


**Figure 2. AdnaTest OvarianCancerDetect: Multiplex PCR of various cancer associated tumor markers.** In a second step the enriched cells are examined by RT-PCR for tumor associated expression patterns. The mRNA strands are reverse transcribed into cDNA. Subsequently, several associated tumor markers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

The 2 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. Make sure to 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 (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 OvarianCancerDetect

### Equipment

- Tube rotator for 1.5 ml tubes
- Magnetic particle concentrator AdnaMag-S
- 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
- 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, 2.500 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 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 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

- 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 applies.
- 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 suppliers other than those 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 the AdnaTest Lysis/Binding Buffer is equilibrated to room temperature (15–25°C). If 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.

   **Important**: Do not vortex.

2. Calculate the volume of OvarianSelect 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.

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).
6. Remove the tube from the AdnaMag-S rack, 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 (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 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 downwards 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.
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 with 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.
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 each supernatant (cell lysate) into a new 1.5 ml reaction tube (provided).
16. Discard the tubes that contain 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 –30 to –15°C for up to 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.

   **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 has to be carried out carefully (watch the pellet), because the beads might 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 29.5 µl 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: 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 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 | 4.0 µl |
| dNTP Mix (5 mM each dNTP) | 4.0 µl |
| RNase inhibitor, 40 U/µl (Promega) | 0.5 µl |
| Sensiscript Reverse Transcriptase | 2.0 µl |
| **Template RNA*** mRNA/bead complex or RNase-free water | 29.5 µl |
| **Total volume** | **40.0 µl** |

* As RT control, add 29.5 µl 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.

2. Vortex the RT Master Mix. Centrifuge briefly, and pipet 10.5 µl 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.

4. The 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 –30 to –15°C for a maximum of 4 weeks.
6. Continue with “Protocol: Multiplex and Duplex PCR and Fragment Analysis”, page 22.

# 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, 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. Prepare the PCR Master Mix 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.

**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*<br>RT control *or*<br>Negative control (RNase-free water) *or*<br>AdnaTest Positive Control Ovarian, each: | 8.0 µl |
| **Total volume** | **50.0 µl** |

2. For each preparation, dispense 42.0 µl of the Master Mix into 0.2 ml PCR reaction tube. 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.

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 37 cycles.

**Table 4. PCR cycling program**

| Step | Time | Temperature |
|---|---|---|
| **Initial activation step** | 15 min | 95°C |
| **3-step cycling (37 cycles)** | | |
| 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. Prepare the PCR Master Mix 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 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**: For 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*<br>RT control *or*<br>Negative control (RNase-free water) *or*<br>AdnaTest Positive Control ERCC1, each: | 8.0 µl |
| **Total volume** | **50.0 µl** |

3. 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**

| Step | 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** | ∞ | 12°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 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. 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 OvarianDetect

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

If you are using the Agilent 2100 Bioanalyzer, peaks with a concentration ≥0.15 ng/µl 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.

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

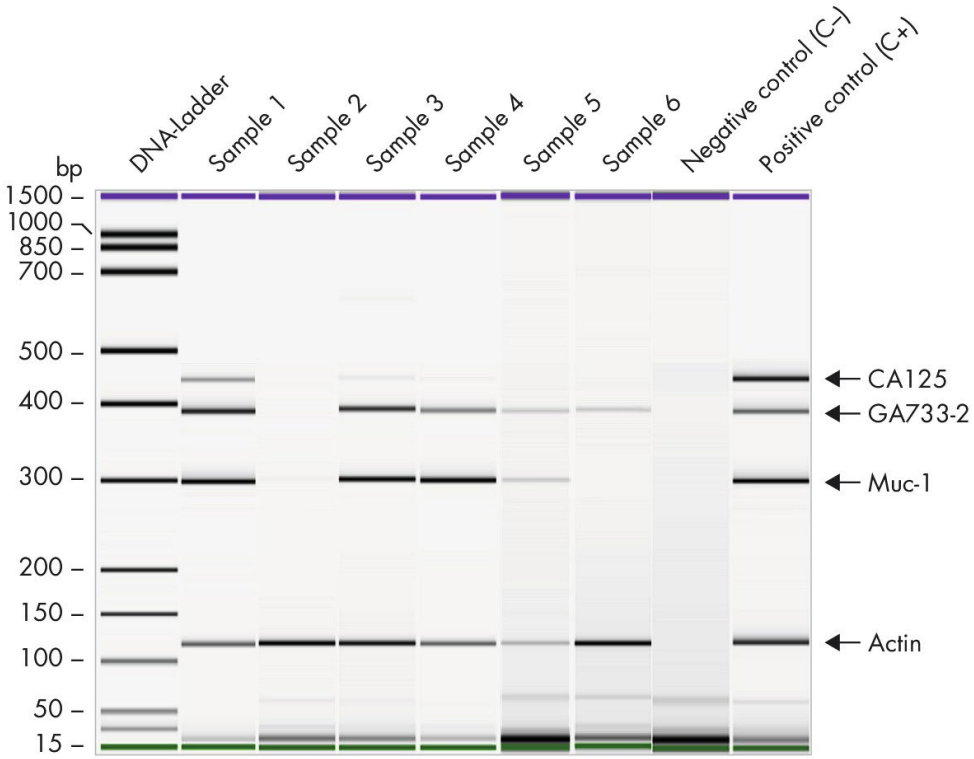


**Figure 3. AdnaTest OvarianCancerDetect results of multiplex PCR samples analyzed with an Agilent 2100 Bioanalyzer.** The first lane shows the DNA size standard (DNA-Ladder). Sample 1 is positive for GA733-2, Muc-1 and CA125; samples 3, 4 and 5 are positive for GA733-2 and Muc-1; and sample 6 is positive for GA733-2. Sample 2 is negative. Actin is detected in samples 1–6. The PCR negative control and positive control are shown in the last 2 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/µl for ERCC1 are positive (Figure 4).

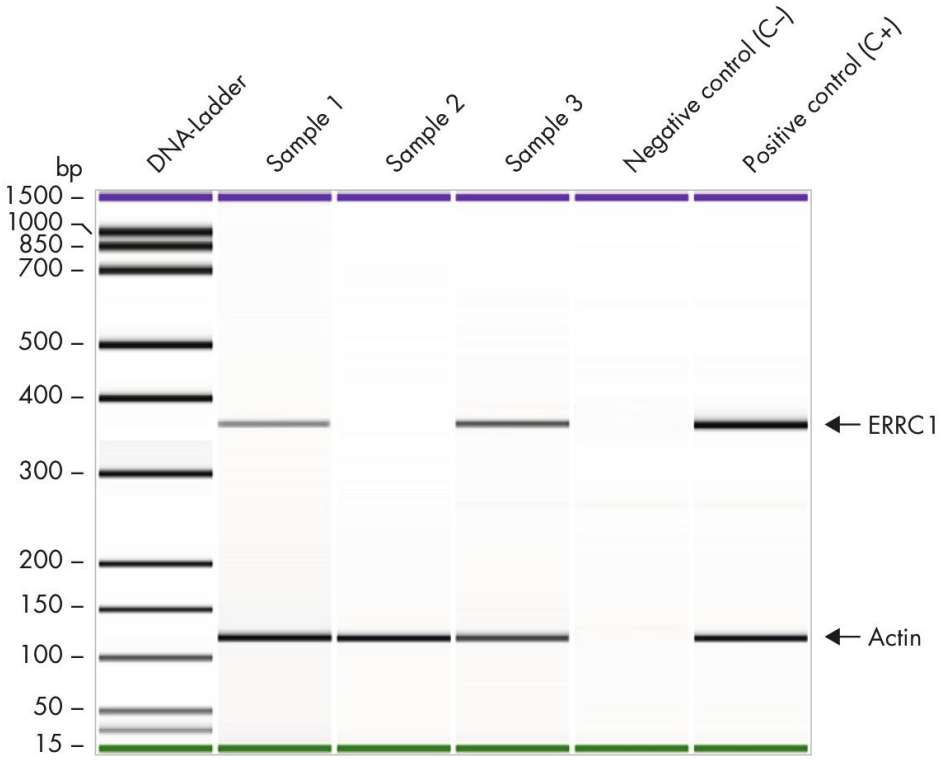


**Figure 4. AdnaTest OvarianCancerDetect results of duplex PCR samples.** The first lane shows the DNA size standard (DNA-Ladder). Samples 1 and 3 are positive for ERCC1. Sample 2 is negative. Actin is detected in samples 1–3. The PCR negative control and positive control (ERCC1) are shown in the last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

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).

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.

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

# 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 | 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 OvarianCancerDetect, 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 OvarianCancerDetect 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 OvarianCancerDetect, because that patent has expired. In "Analysis of the results for AdnaTest ERCC1-Detect", added statement that a fragment larger than 1000 bp indicates contamination with genomic DNA. In "Sample preparation", changed blood storage temperature to 2–8°C, from 4°C. |

HB-2337-002 04/2020