

Version 150715 EN

AdnaTest EMT-2/StemCell Add-on Ovarian-2 Detect

**PCR-expression analysis of ovarian cancer associated gene
expression in enriched tumor cells**

For research use only

Manual

REF T-1-537-PO-2

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Order Information

On the website www.adnagen.com the addresses of distributors and information about our products can be found. Our distributors will provide you also with technical support.






Furthermore, the QIAGEN Hannover support team will answer you any questions regarding the *AdnaTests* (support@adnagen.com).

Purpose

AdnaTest EMT-2/StemCell Add-on Ovarian-2 Detect is used for the analysis of ovarian cancer-associated gene expression in immunomagnetically enriched tumor cells by reverse transcription and PCR and is intended for research use only. The specificity of the detection is 90%. In spiking experiments 5 tumor cells per sample are detected at a recovery rate of at least 90%.

AdnaTest EMT-2/StemCell is used for the enrichment of circulating tumor cells from peripheral blood and reverse transcription.

Abbreviations and Symbols

bp	Base pairs
cDNA	Complementary deoxyribonucleic acid
C+	Positive control
C-	Negative control
CA-125	Cancer antigen 125
DNA	Deoxyribonucleic acid
GA733-2	Gastrointestinal tumor associated antigen 733-2
Muc-1	Muc-1 gene
PCR	Polymerase chain reaction
RNase	Ribonuclease
rpm	Revolutions per minute
RT	Reverse transcription
	Expiry date
	Storage temperature
	Catalogue number
	Consider instructions for use
	Manufactured by

Patents and Registered Trademarks

This test requires licenses of Hoffmann-La Roche AG, Basel. The purchase of *AdnaTests* does not authorize the user to perform the PCR without license. The trademark *HotStarTaq* is registered by QIAGEN, Hilden. *LabChip* is a US registered trademark of Caliper Technology Corp.

Product Description

The *PrimerMix Ovarian-2 Detect* allows the amplification of three tumor associated antigens and one control gene. The primers generate fragments of the following sizes:

CA125: 432 bp

GA733-2: 395 bp

Muc-1: 299 bp

Actin: 120 bp (internal PCR control)

Note: Fragment sizes may vary slightly. Please use the *Positive Control (C+)* [9] for assignment of the detected signals.

Kit Components

AdnaTest EMT-2/StemCell Add-on Ovarian-2 Detect includes the following components:

Table 1: Kit components

Component	Symbol	T-1-537-PO-2 (12 tests)
<i>PrimerMix Ovarian-2 Detect</i>	[8]	1
<i>Positive Control Ovarian-2 (C+)</i>	[9]	1

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

Additional Materials Needed

Equipment:

- Thermocycler with a heated lid and a heating rate of 2 °C/s.
- Agilent 2100 Bioanalyzer (Agilent Technologies) or an alternative analysis system.

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)
- Pipets and RNase-free pipet tips with aerosol barrier, suitable for pipetting volumes from 4 µl to 200 µl
- Protective gloves

Reagents:

- *HotStarTaq Master Mix* Kit (QIAGEN, cat no. 203443, 250 U)

Storage

AdnaTest EMT-2/StemCell Add-on Ovarian-2 Detect has to be stored at -20 °C. In order to prevent possible contaminations and frequent temperature changes aliquot the primer mix. All components must not be used beyond the expiry date.

Application Information

- The test must be performed by personnel skilled in molecular biological techniques.
- All components and additional reagents provided by other suppliers have to be stored according to their instructions. Safety advices of the respective manufacturers are valid.
- Wear protective gloves to avoid contamination with DNA, RNA and RNases.



The test has to be performed in the denoted sequence and has to comply with all specifications stated in respect of incubation times and incubation temperatures.

- Perform sample processing incl. reverse transcription (*AdnaTest EMT-2/StemCell*) 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 cause inferior results.**
- The safety and hygiene regulations of the laboratory must be respected (e. g. wear lab coats, protective goggles, gloves).

Protocol

A. Multiplex-PCR

1. Thaw *HotStarTaq Master Mix* (QIAGEN), RNase-free water, *Positive Control Ovarian-2 (C+)* [9] and *PrimerMix Ovarian-2 Detect* [8], vortex, spin down and place on ice.
2. The PCR Master Mix is prepared as shown in Table 2 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 a *Positive Control Ovarian-2 (C+)* [9], RNase-free water as Negative Control (C-) and the RT Control must always be included.

3. 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 2: Preparation of the Multiplex-PCR

Component		Volume
PCR Master Mix	<i>HotStarTaq Master Mix</i>	25.0 µl
	RNase-free water	13.0 µl
	<i>PrimerMix Ovarian-2 Detect</i> [8]	4.0 µl
Samples	cDNA or RT Control or Negative Control (RNase-free water) or <i>Positive Control Ovarian-2 (C+)</i> [9] each:	8.0 µl
Total volume		50.0 µl

A thermocycler is used for the PCR following the program described in Table 3. Run the thermocycler with a ramp of 2 °C/second. The PCR is performed with a total of 37 cycles.

Table 3: PCR program

95 °C	15min	} 37 cycles
94 °C	30sec	
58 °C	30sec	
72 °C	30sec	
72 °C	10 min	
4 °C	∞	

B. Fragment Analysis

Agilent 2100 Bioanalyzer

The analysis with the Agilent 2100 Bioanalyzer (Agilent Technologies) on a DNA 1000 LabChip is recommended. 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.

Start the Bioanalyzer software "2100 expert". Under "Contexts" select "Instrument", click the button "Assay" next to "Assay selection". Choose "electrophoresis > DNA 1000 Series II.xsy". Prepare the chip and start run.

For evaluation of the results set a detection threshold as it is described below:

Under "Contexts" select "Data", choose the tab "Assay Properties". On the right select "Global" and "Normal" from the pull down menu. Choose "Sample Setpoints > Integrator > height threshold (FU)" and set this value to "0" (default value is "20") to detect all signals.

Evaluation

Using the Agilent 2100 Bioanalyzer, peaks with a concentration of ≥ 0.20 ng/ μ l are positive (Fig. 1).

The fragment of the control gene actin must show in all patient samples (internal PCR control). An actin signal provides a positive control for the Multiplex-PCR. Negative Control and RT Control samples must not show any bands larger than 80 base pairs (primer dimers).

Any deviation from the protocol might lead to false negative or false positive results.

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

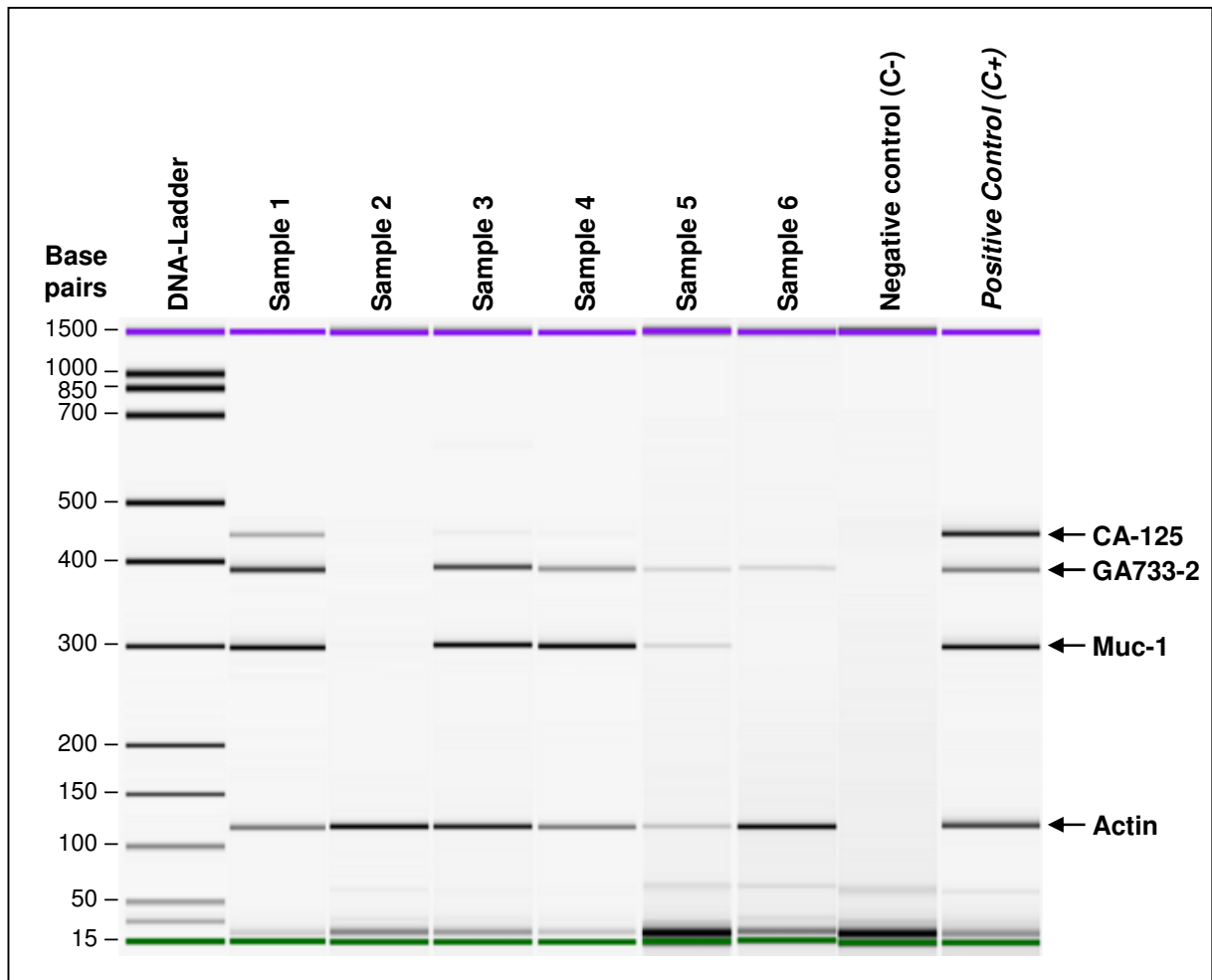


Fig. 1: AdnaTest EMT-2/StemCell Add-on Ovarian-2 Detect results of multiplex-PCR samples analyzed with an Agilent 2100 Bioanalyzer

The first lane shows the DNA size standard (DNA-Ladder). Sample 1 is positive for CA-125, GA733-2 and Muc-1, 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 (C-) and *Positive Control (C+)* are shown in the last two lanes.

References

For references please refer to our website

<http://www.adnagen.com>

Troubleshooting

A failure of the gene expression analysis may have various reasons. It is essential that all assay steps are always executed precisely according to the manual. In case problems still occur, please go to: www.adnagen.com and download our troubleshooting guide in the product section. You will find practical hints for the test procedure and for the correct interpretation of test results.

Do not hesitate to contact our support team when problems continue to exist.

Short Manual

AdnaTest EMT-2/StemCell Add-on Ovarian-2 Detect

Components	<i>PrimerMix Ovarian-2 Detect</i>	8
	<i>Positive Control Ovarian-2 (C+)</i>	9
You need	<ul style="list-style-type: none"> • 0.2 ml PCR-tubes • pipets and tips (RNase free) for 4 - 200 µl • <i>HotStarTaq Master Mix</i> Kit (QIAGEN) 	

Thaw all components, mix and keep on ice before use.

Table 4: Multiplex-PCR

Component		Volume
PCR Master Mix	<i>HotStarTaq Master Mix</i>	25.0 µl
	RNase-free water	13.0 µl
	<i>PrimerMix Ovarian-2 Detect</i> 8	4.0 µl
Samples	cDNA or RT Control or Negative Control (RNase-free water) or <i>Positive Control Ovarian-2 (C+)</i> 9 each:	8.0 µl
Total volume		50.0 µl

- The PCR is performed with a total of 37 cycles.

Table 5: PCR program

95 °C	15 min	} 37 cycles
94 °C	30 sec	
58 °C	30 sec	
72 °C	30 sec	
72 °C	10 min	
4 °C	∞	

- For fragment analysis we recommend the use of an Agilent 2100 Bioanalyzer.



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