

Version 150715 EN

# *AdnaTest EMT-2/StemCell Add-on ColonDetect*

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

*For research use only*

## **Manual**

**REF** T-1-537-PC

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## Order Information

On the website [www.adnagen.com](http://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 ColonDetect* is used for the analysis of colon 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                                |
| CEA   | Carcinoembryonic antigen                        |
| DNA   | Deoxyribonucleic acid                           |
| EGFR  | Epidermal growth factor receptor                |
| GA733-2   | Gastrointestinal tumor associated antigen 733-2 |
| 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 ColonDetect* allows the amplification of three tumor associated antigens and one control gene. The primers generate fragments of the following sizes:

|          |                               |
|----------|-------------------------------|
| GA733-2: | 395 bp                        |
| CEA:     | 231 bp                        |
| EGFR:    | 163 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 ColonDetect* includes the following components:

**Table 1: Kit components**

| Component                          | Symbol | T-1-537-PC<br>(12 tests) |
|------------------------------------|--------|--------------------------|
| <i>PrimerMix ColonDetect</i>       | [8]    | 1                        |
| <i>Positive Control Colon (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 ColonDetect* 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 Colon (C+)* [9] and *PrimerMix ColonDetect* [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 Colon (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 ColonDetect</i> [8]   | 4.0 µl         |
| Samples             | cDNA or<br>RT Control or<br>Negative Control (RNase-free water) or<br><i>Positive Control Colon (C+)</i> [9] each: | 8.0 µl         |
| <b>Total volume</b> |  | <b>50.0 µl</b> |



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 38 cycles.

**Table 3: PCR program**

|       |       |             |
|-------|-------|-------------|
| 95 °C | 15min | } 38 cycles |
| 94 °C | 45sec |             |
| 58 °C | 45sec |             |
| 72 °C | 45sec |             |
| 72 °C | 10min |             |
| 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**

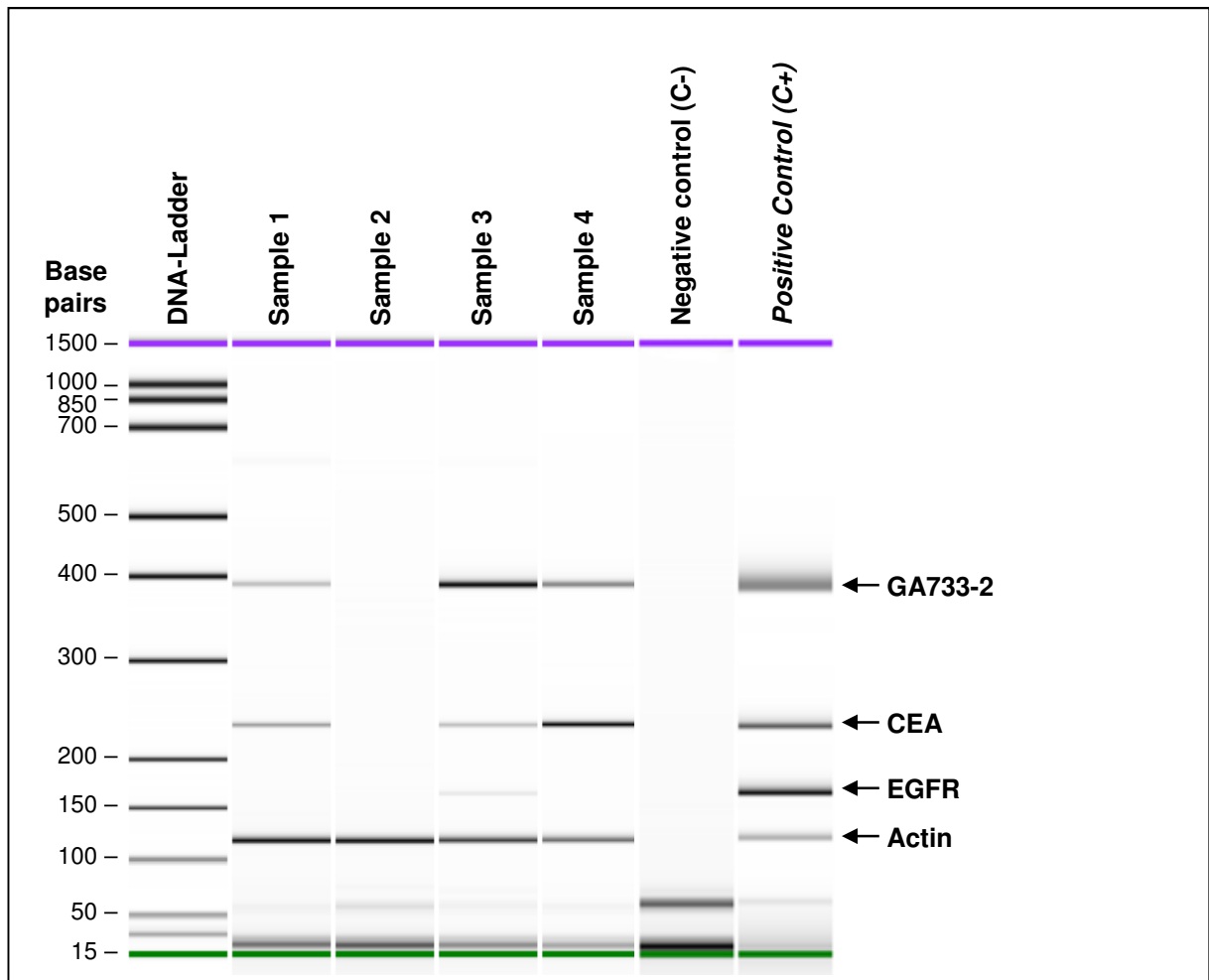
Using the Agilent 2100 Bioanalyzer, peaks with a concentration of  $\geq 0.10$  ng/ $\mu$ l are positive (Fig. 1).

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).

A fragment larger than 900 bp indicates a contamination with genomic DNA. The separation process was not successful and the results have to be discarded if such a fragment is detected.

**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 ColonDetect results of samples analyzed with an Agilent 2100 Bioanalyzer**

The first lane shows the DNA size standard (DNA-Ladder). Sample 1 and 4 are positive for GA733-2 and CEA, sample 2 is negative and sample 3 is positive for GA733-2, CEA and EGFR. Actin is detected in samples 1 to 4. 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](http://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 ColonDetect*

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

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

**Table 4: Multiplex-PCR**

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

- The PCR is performed with a total of 38 cycles.

**Table 5: PCR program**

|       |       |             |
|-------|-------|-------------|
| 95 °C | 15min | } 38 cycles |
| 94 °C | 45sec |             |
| 58 °C | 45sec |             |
| 72 °C | 45sec |             |
| 72 °C | 10min |             |
| 4 °C  | ∞     |             |

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





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