



AdnaTest ER/PR-Detect

PCR-expression analysis of hormone receptor genes for estrogen and progesterone in enriched tumor cells

For in-vitro diagnostic use

Manual



Article no. T-1-532

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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, AdnaGen's support team will answer you any questions regarding the *AdnaTests* (support@adnagen.com).

AdnaTest ER/PR-Detect can be ordered as listed below.

	Specifications	Order no.
<i>AdnaTest ER/PR-Detect</i>	12 Selections	T-1-532



Purpose

AdnaTest ER/PR-Detect for the analysis of estrogen and progesterone hormone receptor genes expression in immunomagnetically enriched tumor cells by reverse transcription and PCR is intended for *in-vitro* diagnostic use only.

The *AdnaTest BreastCancerSelect* is recommended for the enrichment of circulating tumor cells.

Further information can be found on the website www.adnagen.com.

Abbreviations and Symbols

bp	base pairs
cDNA	complementary deoxyribonucleic acid
C+	positive control
C-	negative control
DNA	deoxyribonucleic acid
ER	Estrogen
PR	Progesterone
PCR	polymerase chain reaction
rpm	revolutions per minute
RT	reverse transcription
	expiry date
	storage temperature

Patents and Registered Trademarks

This test requires licenses of Hoffmann-La Roche AG, Basel. The purchase of *AdnaTests* does not relieve 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

With the *PrimerMix ER/PR-Detect* the hormone receptor genes for estrogen, progesterone and one control gene, actin, are amplified. The primers generate fragments of the following sizes:

ER : 305bp
PR : 217 bp
Actin : 119 bp (internal PCR control).

Kit Components

AdnaTest ER/PR-Detect includes the following components:

Table 1: Kit components

Component	Symbol	(12 tests)	(24 tests)	(36 tests)
<i>PrimerMix ER/PR-Detect</i>	1	1	2	3
<i>Positive Control (C+)</i>	2	1	2	3
<i>Gel Calibrator</i>	3	1	2	3

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

Additional Materials Needed

Equipment:

- Agarose gel electrophoresis and image documentation system or an alternative analysis system like the Bioanalyzer Agilent 2100 (Agilent Technologies).
- Thermocycler

Material:

- Sterile, RNase-free thin-wall 0.2 ml PCR-tubes
- Pipets (0.5- 200 µl), RNase-free pipet tips with aerosol barrier
- Protective gloves

- Agarose gels, for instance a precast 4 % agarose gel containing ethidium bromide (SIGMA, cat no. P 6097)

Reagents:

- HotStarTaq Master Mix Kit (Qiagen, e. g. cat no. 203443, 250 U)

Storage

AdnaTest ER/PR-Detect has to be stored at -20 °C. In order to prevent possible contaminations and frequent temperature changes aliquot the primer mix and the gel calibrator. 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 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 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) and *Positive Control (C+)* [2], vortex carefully, centrifuge quickly and store on ice. Thaw *PrimerMix ER/PR-Detect* [1], 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 (C+)* [2], a Negative Control (water) 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 the cDNA.

Note: 8.0 µl of distilled water is added instead of cDNA as negative control.

Table 2: Preparation of the multiplex PCR

Components		Volumes
PCR Master Mix	HotStarTaq Master Mix	25.0 µl
	Distilled water	13.0 µl
	<i>PrimerMix ER/PR-Detect</i> [1]	4.0 µl
Samples	cDNA or RT Control or Negative Control (water) or <i>Positive Control (C+)</i> [2] 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.

Table 3: PCR program

95 °C	15 min	} 37 cycles
94 °C	30 sec	
60 °C	30 sec	
72 °C	30 sec	
72 °C	5 min	
4 °C	∞	

B Fragment Analysis

Agilent Bioanalyzer2100

The analysis with the Agilent Bioanalyzer2100 (Agilent Technologies) on a DNA 1000 LabChip is recommended. Carry out the instructions of the DNA 1000 LabChip manual. When using the Agilent Bioanalyzer2100 set a detection threshold as it is described below:

Start the Bioanalyzer Software Bio Sizing and create a Default Assay. Under Instrument select “Assay > Electrophoresis > ds1000 > DNA 1000 Series II”. Under “Data” choose “Assay properties > global normal > height threshold (FU)” set the “Min Peak Height” to “0” to detect all signals.

Agarose Gel

Alternatively, the PCR products are analyzed by electrophoresis on a 4 % agarose gel. Apply 10.0 µl of each product and 10.0 µl of the *Gel Calibrator* [3]. Include a 100 bp DNA ladder as size marker according to the manufacturer’s instructions.

To make sure that the fragments can be discriminated accurately run the gel over a distance of at least 5 cm. Electrophoresis conditions: 100 V, ≥ 1 h.

Evaluation

If you are using the Agilent Bioanalyzer2100, peaks with a concentration of ≥ 0.15 ng/µl for ER and with a concentration of > 0 ng/µl for PR are positive.

PR is considered positive in a agarose gel if there is a signal. For the evaluation of ER in a agarose gel the *Gel Calibrator* [3] helps to evaluate the agarose gel. ER fragments that are stained more intensive than the the banding in *Gel Calibrator* [3] are positive; those with a weaker stain are negative.

In addition, the following criteria must be fulfilled:

- 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. The actin signal may be very weak if the ER and PR signals are very strong.
- The Negative Control and the RT Control samples must not show any bands larger than 80 base pairs (primer dimers).

Note: The *AdnaTest ER/PR-Detect* is optimized to exclude illegitimate expression of the ER and PR transcripts. Any change in the protocol might lead to loss of specificity.

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

In the case that assistance is needed to interpret the result, please, contact our support team.

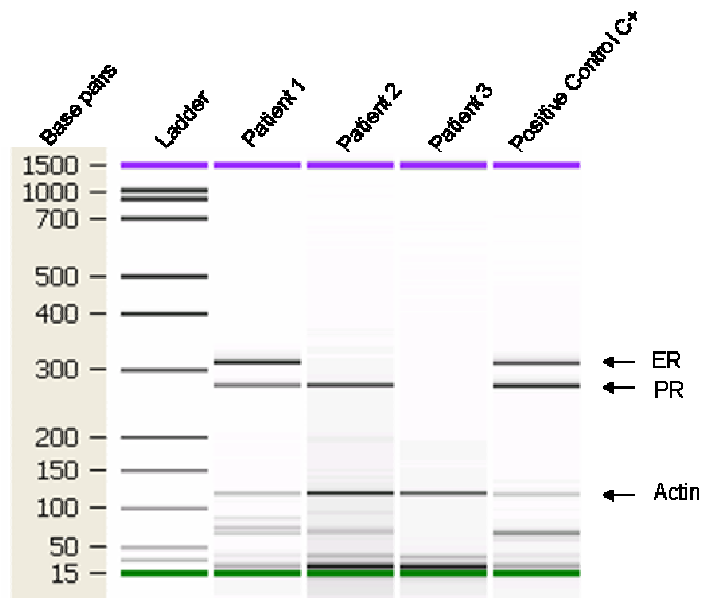


Fig. 1: AdnaTest ER/PR-Detect positive samples: Patient 1 and Positive Control expression products of estrogen (305 bp), progesterone (270 bp) and as internal control actin (119 bp) analyzed with the Bioanalyzer2100 (Agilent). Patient 2 is positive for PR and actin and patient 3 for actin only.

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 that there are still problems, the following table gives you comments on the possible causes and suggests corrections. Do not hesitate to contact our support team when problems continue to exist.

Table 4: Troubleshooting

Problem	Possible causes	Suggestions for correction
No bands incl. actin, for all samples	Pipetting error	Repeat test
	Reagents problems	Control reagents (storage etc.).
	RNase contamination	Use only RNase-free pipets, tips, reaction tubes and reagents. Wear gloves and change them regularly.
	Band could not be identified because of insufficient separation.	Check gel concentration, buffers, separation time and the applied voltage.
RT and C-controls show fragments larger than 80 bp	Contamination	Exchange all reagents. Aliquot all reagents before use. Use filter tips. If possible, keep the preparation of samples and the reaction setup locally separated from the analysis of PCR products.
Diffuse bands in the agarose gel	Gel electrophoresis conditions are not optimal.	Check concentration of agarose gel. Check electrophoresis buffer.

Short Manual

AdnaTest ER/PR-Detect

Component	<i>PrimerMix ER/PR-Detect</i>	1
	<i>Positive Control (C+)</i>	2
	<i>Gel Calibrator</i>	3
You need	0.2 ml PCR-tubes 0.5-200 µl pipets and tips (RNase free) 4 % Agarose gel HotStarTaq Master Mix Kit (Qiagen).	

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

Table 5: Multiplex PCR

Components		Volumes
PCR Master Mix	HotStarTaq Master Mix	25.0 µl
	Distilled water	13.0 µl
	<i>PrimerMix ER/PR-Detect</i> 1	4.0 µl
Samples	cDNA or RT Control or Negative Control (water) or <i>Positive Control (C+)</i> 2 each:	8.0 µl
Total volume		50.0 µl

Table 6: PCR program

95 °C	15 min	} 37 cycles
94 °C	30 sec	
60 °C	30 sec	
72 °C	30 sec	
72 °C	5 min	
4 °C	∞	

- For fragment analysis use the Bioanalyzer 2100 (Agilent). Alternatively analyze the fragments, the samples and the *Gel Calibrator* ³ in a 4 % agarose gel (100 V for ca. 60 min).

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