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For Professional Use Only

eSens BRCA1 SNP PCR kit

REF ES4200B

Instructions for Use

1 INTENDED USE

eSens BRCA1 SNP PCR kit is an *in vitro* nucleic acid amplification test for determination of genetic susceptibility to cancer by qualitative detection of germline mutations (5382insC (rs80357906), 4153delA (rs80357711), 300T>G (C61G) (rs28897672) and 185delAG (rs80357914)) in the *BRCA1* gene in biological material (venous whole blood), using real-time hybridization-fluorescence detection of amplified products. The material for PCR is DNA samples extracted from test material.

Indications and contra-indications for use of the reagent kit

The reagent kit is used in clinical laboratory diagnostics and can be applied in medical practice for determination of genetic susceptibility to cancer associated with mutations in the *BRCA1* gene, without distinction of presence of manifestation, as well as to confirm the diagnosis of hereditary breast cancer or ovarian cancer.

A negative test result does not rule out the presence of other mutations in the *BRCA1* gene and other genes, or the general population risk of cancer.

There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

The reagent kit can be used in medical practice for examination of individuals of either gender who belong to the Caucasian race.

NOTE: The results of PCR analysis are taken into account in complex diagnostics of disease.

2 PRINCIPLE OF PCR DETECTION

Principle of testing is based on amplification of human genomic DNA fragments including 5382insC (rs80357906), 4153delA (rs80357711), 300T>G (C61G) (rs28897672) and 185delAG (rs80357914) mutations in the *BRCA1* gene with hybridization-fluorescence detection.

The *BRCA1* gene fragment with no mutation at position 5382 (5382 WT) is used as an endogenous internal control. The functional copy of the *BRCA1* gene must always be present in the test sample in a

sufficient amount proportional to the number of human cells in the sample. Endogenous internal control allows not only to control all stages of the PCR analysis for each sample, but also to evaluate the adequacy of the material sampling and its storage.

Amplification of DNA fragments with the use of specific primers and Taq-polymerase enzyme are performed with the DNA samples obtained at the extraction stage. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

eSens BRCA1 SNP PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

eSens BRCA1 SNP PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate (dUTP).

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	**FAM**	**JOE**	**ROX**	**Cy5**	**Cy5.5**
DNA-target	5382insC (rs80357906)	4153delA (rs80357711)	300T>G (rs28897672)	185delAG (rs80357914)	5382 WT (no mutation)
Target gene	*BRCA1*	*BRCA1*	*BRCA1*	*BRCA1*	*BRCA1*

3 CONTENT

eSens BRCA1 SNP PCR kit (ES4200B) includes:

Reagent	**Description**	**Volume, ml**	**Quantity**
PCR-mix-FL BRCA1	clear liquid from colorless to blue grey colour	0.6	1 tube
PCR-buffer-C	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
C+ BRCA WT	colorless clear liquid	0.2	1 tube
C+ BRCA Het	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.2	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube

***** must be used in the extraction procedure as Negative Control of Extraction.

eSens BRCA1 SNP PCR kit is intended for 55 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

For sampling and pretreatment

- Reagent for pretreatment of whole peripheral and umbilical blood.
- Vacuum tubes for sampling, storage and transportation of blood samples.
- Sterile bilateral needles for vacuum tubes intended for venous blood collection.
- Disposable screwed or tightly closed polypropylene 1.5-ml tubes.
- Sterile pipette tips with aerosol filters (up to 100, 200 and 1000 µl).
- Tube racks.
- PCR box.
- Desktop centrifuge up to 12,000 g (suitable for Eppendorf tubes).
- Vortex mixer.
- Vacuum aspirator with flask for removing supernatant.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir to throw off and inactivate the material.
- Disposable powder-free gloves and a laboratory coat.

For DNA extraction and amplification

- DNA extraction kit.
- TE-buffer reagent, 5-ml tube.
- Sterile pipette tips with aerosol filters (up to 100, 200 and 1000 µl).
- Tube racks.
- Vortex mixer.
- PCR box.
- Real-time instruments (for example, Rotor-Gene Q (QIAGEN, Germany), CFX 96 Touch, CFX 96 Opus (Bio-Rad, USA), QuantStudio 5 (Thermo Fisher Scientific), or equivalent)).
- Disposable polypropylene PCR tubes:
  a) screwed or tightly closed 1.5-ml tubes for reaction mixture preparation.
  b) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
  c) thin-walled 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves and a laboratory coat.

5 GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.

- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section “Content”).
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

⚠ Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6 SAMPLING AND HANDLING

eSens BRCA1 SNP PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the biological material (venous whole blood).

Sampling

6.1 Venous whole blood.

Venous whole blood should be taken with a disposable 0.8-1.1 mm diameter needle into the tube (special vacuum system) with EDTA as anticoagulant. After blood sampling the tube should be smoothly rotated several times for the thoroughly mixing with the anticoagulant.
(Otherwise, blood will coagulate and DNA extraction will be impossible!) Place the tube in the rack after rotating.
Venous whole blood samples can be stored before pretreatment:

- at the temperature from 20 to 25 °C – for 6 hours after sampling of biological material;
- at the temperature from 2 to 8 °C – for 1 day.

Freezing of venous whole blood samples is unallowable!

Venous whole blood can be transported at 2–8 °C for 1 day.

Pretreatment

Venous whole blood is to be pretreated.

Transfer 1.0 ml of Hemolytic and 1.0 ml of venous whole blood to the 1.5-ml Eppendorf tube. Gently vortex the tube and leave it for 10 min, stirring occasionally. Centrifuge at 8,000 rpm for 2 min. Remove the supernatant using vacuum aspirator without touching the pellet. After washing the cell pellet should be white, only a small pinkish bloom on the pellet is allowed (the remains of the destroyed erythrocytes). The washing using Hemolytic may be repeated if necessary. The obtained leukocyte pellet must be immediately lysed (in case of extraction using **RIBO-prep** reagent kit (K2-9-Et-100-CE) add **300 µl** of **Solution for Lysis** and then extract DNA in accordance with the *Instruction Manual* enclosed to the **RIBO-prep** reagent kit without adding **Solution for Lysis** once again) or frozen.
The frozen pellet can be stored at the temperature from minus 24 to minus 16 °C for 2 weeks or at the temperature not more than minus 68 °C for 1 year.
In case of using other reagent kits for DNA extraction the test material should be pretreated if necessary in accordance with the *Instruction Manual* to the using reagent kit.

Interfering substances and limitations of using test material samples

- The next samples are inapplicable for analysis:
- the venous whole blood samples, collected in the tubes with heparin as anticoagulant,
- the venous whole blood samples, containing blood clot or which has been exposed to freezing.

Potential interfering substances

Endogenous and exogenous substances that may be present in the biological material (venous whole blood) used for the study were selected to assess potential interference.
Venous whole blood samples with known genotypes without adding and with the addition of potential interfering substances were tested. Concentration of each potential interfering substance is specified in the Table 2. Each sample was tested in three repeats. Genotype of venous whole blood samples was confirmed by pyrosequencing with PyroMark series genetic analysis system.

Table 2

Type of potential interferent	Potential interferent	Tested concentration	Interference presence
Exogenous substances	Potassium EDTA	up to 2.0 mg/ml	Not detected
	Lithium heparin	from 12 IU/ml	Detected
Endogenous substances	Total bilirubin	210 µmol/l (upper limit of normal – 21 µmol/l)	Not detected
	Total cholesterol	78 mmol/l (upper limit of normal – 7.8 mmol/l)	Not detected
	Triglycerides	37.0 mmol/l (upper limit of normal – 3.7 mmol/l)	Not detected
	Hemoglobin	250 g/l (upper limit of normal – 170 g/l)	Not detected

7 WORKING CONDITIONS

eSens BRCA1 SNP PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8 PROTOCOL

8.1 DNA extraction

Any commercial nucleic acid extraction kit, if IVD-CE validated for the indicated specimen types, could be used.

Ecoli Dx, s.r.o. recommends:

- For the manual extraction

- **RIBO-prep** (K2-9-Et-100-CE)

- For the automatic extraction

- **ePure Blood DNA Extraction Kit** (E2001)

NOTE: Extract the DNA according to the manufacturer's protocol.

8.2 Preparing PCR

8.2.1 Preparing tubes for PCR

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

The total reaction volume is **25 µl**, the volume of the **DNA** sample is **10 µl**.

It is recommended to determine DNA concentration in obtained extracted samples and to dilute extracted DNA using TE-buffer to required concentration 10-30 ng per reaction (corresponds to concentration of extracted DNA $3x10^5$–$9x10^5$ copies/m) (it is recommended to use TE-buffer reagent manufactured by FBIS CRIE).

1. Calculate the required quantity of each reagent for reaction mixture preparation. For one reaction:

   10 µl of **PCR-mix-FL BRCA1,**
   5 µl of **PCR-buffer-C,**
   0.5 µl of **polymerase (TaqF)**.

Prepare the reaction mixture for the total number of test and control samples plus some extra reactions. See numbers of control samples in item 7.

NOTE: Prepare the reaction mixture just before use.

2. Thaw the tube with **PCR-mix-FL BRCA1**. Mix the content of the tubes with **PCR-mix-FL BRCA1, PCR-buffer-C** and **polymerase (TaqF)**. Sediment the drops by vortex.
3. In a new tube prepare the reaction mixture. Mix the required quantities of **PCR-mix-FL BRCA1, PCR-buffer-C** and **polymerase (TaqF)**. Sediment the drops by vortex.

NOTE: The prepared mixture should be stored for no more than 2 hours.

4. Take the required number of the tubes or strips for PCR of DNA of test and control samples.

5. Transfer **15 µl** of the prepared reaction mixture to each tube. Discard the unused reaction mixture.
6. Transfer to the prepared tubes **10 µl** of **DNA samples** obtained by extraction from the test samples and diluted to the optimal concentration.
7. Carry out the control amplification reactions:

C+ WT	–	Add **10 µl** of **C+ BRCA WT** to the tube labeled C+ WT (Positive Control of Amplification)
C+ Het	–	Add **10 µl** of **C+ BRCA Het** to the tube labeled C+ Het (Positive Control of Amplification)
NCA	–	Add **10 µl** of **TE-buffer** to the tube labeled NCA (Negative Control of Amplification)
C–	–	Add **10 µl** of **the sample extracted from the Negative Control (C–) reagent** to the tube labeled C– (Negative control of Extraction).

8.2.2 Amplification

1. Create a temperature profile on your instrument as follows:

Table 3

eSens-1 amplification program

Rotor-type Instruments (e.g Rotor-Gene Q or equivalent)				**Plate-type Instruments** (e.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent)			
Step	**Temperature, °C**	**Time**	**Cycles**	**Step**	**Temperature, °C**	**Time**	**Cycles**
1	**50**	**15 min**	**1**	**1**	**95**	**15 min**	**1**
2	**95**	**15 min**	**1**	**2**	**95**	**10 s**	**45**
3	**95** **60**	**10 s** **20 s**	**45**		**60**	**27 s**	
	95	**10 s**					

NOTE: Any combination of the tests (including tests with reverse transcription and amplification) can be performed in one instrument simultaneously with the use of the unified amplification program for rotor-type instruments. If only the tests for DNA detection are performed in one instrument then the first step of reverse transcription (50 °C – 15 min) can be omitted for time saving.

Fluorescent signal is detected in the channels for the **FAM, JOE, ROX, Cy** and **Cy5** fluorophores.

2. Adjust the fluorescence channel sensitivity.
3. Insert tubes into the reaction module of the device.

NOTE: It is recommended to sediment drops from walls of tubes by short centrifugation before placing them into the instrument.

Insert empty tubes at the edges of reaction module in case of incomplete filling of plate-type instrument.

4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

8.3 Instrument Settings

Test settings for rotor-type instruments

Channel	Calibrate / Gain Optimisation	Dynamic tube	Slope Correct	More Settings/ Outlier Removal
FAM/Green	from 5FI to 10FI	on	on	15 %
JOE/Yellow	from 4FI to 8FI	on	on	30 %
ROX/Orange	from 5FI to 9FI	on	on	15 %
Cy5/Red	from 4FI to 8FI	on	on	20 %
Cy5.5/Crimson	from 4FI to 8FI	on	on	20 %

Test settings for plate-type instruments

Note: Set ***Ramp Rate* 2,5 °C/sec** by clicking the ***Step Options*** button for each step of cycling.

Threshold line settings for plate-type and rotor-type instruments

Channel	Threshold
FAM, **ROX, Cy5**	Set the threshold line at the level corresponding to 10 % of maximum fluorescence level obtained for C+ Het sample
JOE	Set the threshold line at the level corresponding to: • **25 %** of maximum fluorescence level obtained for C+ Het sample for CFX96; • **10 %** of maximum fluorescence level obtained for C+ Het sample Het for other instruments
Cy5.5	Set the threshold line at the level corresponding to **10 %** of maximum fluorescence level obtained for C+ WT sample

9 DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in five channels:

Table 4

Channel for the fluorophore	FAM	JOE	ROX	Cy5	Cy5.5
Amplification product	5382insC (rs80357906)	4153delA (rs80357711)	300T>G (rs28897672)	185delAG (rs80357914)	5382 WT (no mutation)

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid. The fluorescence curves of the sample should once cross the threshold line in the area of typical exponential growth of fluorescence. Characteristics of threshold line settings for plate-type and rotor-type instruments are specified in the chapter 8.3.

Principle of interpretation is the following:

Table 5

Results interpretation

***Ct* value in the channel for the fluorophore**					**Result**
FAM	**JOE**	**ROX**	**Cy5**	**Cy5.5**	
< boundary value	absent or > boundary value	absent or > boundary value	absent or > boundary value	< boundary value	5382insC (rs80357906) mutation **is detected**
absent or > boundary value	< boundary value	absent or > boundary value	absent or > boundary value	< boundary value	4153delA (rs80357711) mutation **is detected**
absent or > boundary value	absent or > boundary value	< boundary value	absent or > boundary value	< boundary value	300T>G (rs28897672) mutation **is detected**
absent or > boundary value	absent or > boundary value	absent or > boundary value	< boundary value	< boundary value	185delAG (rs80357914) mutation **is detected**
absent or > boundary value	absent or > boundary value	absent or > boundary value	absent or > boundary value	< boundary value	Detectable mutations in the *BRCA1* gene **are NOT detected**
determined or absent	determined or absent	determined or absent	determined or absent	absent or > boundary value	**Invalid*** result

***** In case of **invalid** result, the PCR analysis should be repeated for the corresponding test sample starting from the DNA extraction stage. If the same result was obtained once again, re-sampling of the material for analysis is recommended.

NOTE: It is recommended to confirm the result with additional analysis using sequencing methods for the samples with detected mutations. A negative test result does not rule out the presence of other mutations in the BRCA1 gene and other genes, or the general population risk of cancer.

The result of the PCR analysis is considered reliable only if the results obtained for controls of extraction and amplification stages are correct (according to the Table 6, Table 7 and Table 8).

Table 6

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore				
		FAM	**JOE**	**ROX**	**Cy5**	**Cy5.5**
C–	DNA extraction	absent or > boundary value	absent or > boundary value	absent or > boundary value	absent or > boundary value	absent or > boundary value
NCA	PCR	absent or > boundary value	absent or > boundary value	absent or > boundary value	absent or > boundary value	absent or > boundary value
C+ WT	PCR	absent or > boundary value	absent or > boundary value	absent or > boundary value	absent or > boundary value	< boundary value
C+ Het	PCR	< boundary value	< boundary value	< boundary value	< boundary value	< boundary value

Table 7

Boundary Ct values for rotor-type instruments

Sample	Channel for fluorophore				
	FAM	**JOE**	**ROX**	**Cy5**	**Cy5.5**
C+WT	*Ct* value is absent or > 36.5	*Ct* value is absent or> 35.5	*Ct* value is absent or > 35.5	*Ct* value is absent or > 34.5	31
C+ Het	31	30	30	29	31
NCA	*Ct* value is absent or > 36.5	*Ct* value is absent or > 35.5	*Ct* value is absent or > 35.5	*Ct* value is absent or > 34.5	*Ct* value is absent or > 36.5
C-	*Ct* value is absent or > 36.5	*Ct* value is absent or > 35.5	*Ct* value is absent or > 35.5	*Ct* value is absent or> 34.5	*Ct* value is absent or > 36.5
Test samples	36.5	35.5	35.5	34.5	36.5

Table 8

Boundary *Ct* values for plate-type instruments

Sample	Channel for fluorophore				
	FAM	**JOE**	**ROX**	**Cy5**	**Cy5.5**
C+WT	*Ct* value is absent or > 38.5	Ct value is absent or > 37.5	Ct value is absent or > 37.5	Ct value is absent or> 36.5	33
C+ Het	33	32	32	31	33
NCA	*Ct* value is absent or > 38.5	Ct value is absent or > 37.5	Ct value is absent or > 37.5	Ct value is absent or> 36.5	Ct value is absent or > 38.5
C-	Ct value is absent or > 38.5	Ct value is absent or> 37.5	Ct value is absent or > 37.5	Ct value is absent or > 36.5	Ct value is absent or > 38.5
Test samples	38.5	37.5	37.5	36.5	38.5

NOTE: If the *Ct* value determined in the channel for the Cy5.5 fluorophore for the test sample is less than the boundary value and the *Ct* value determined in one or more other detection channels (FAM and/or JOE, and/or ROX, and/or Cy5) is less than the boundary value and the final fluorescence level in this/these channels is significantly lower than the final fluorescence level for C+ Het, it is necessary to evaluate the level of fluorescence of the test sample relative to C+ WT.

Note: For Rotor-Gene Q C+ WT fluorescence graph will appear after ***More Settings/Outlier Removal*** shutdown for the detection channel (FAM and/or JOE, and/or ROX, and/or Cy5) where the final fluorescence level of the test sample is significantly lower than the final fluorescence level of C+ Het.

If the final fluorescence level of the test sample is erose to the fluorescence level of C+ WR, it is assumed that there is no mutation detectable in this channel. The analysls (beginning with the amplification stage) should be repeated for the sample at the optimal concentration (see *Instruction manual, 8.2. Preparing PCR).*

10 TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. The *Ct* value determined for the Positive Control of Amplification (C+ Het) is greater than the boundary value or absent in any of the specified channels for fluorophores (see Table 6). The amplification and detection should be repeated for all samples.
2. The *Ct* value determined for the Positive Control of Amplification (C+ WT) is greater than the boundary value or absent in the channel for the Cy5.5 fluorophore. The amplification and detection should be repeated for all samples.
3. If the *Ct* value determined for the Negative Control of Extraction (C–) and Negative Control of amplification (NCA) is less than the boundary value in the channels for the FAM and/or JOE, and/or ROX, and/or Cy5, and/or Cy5.5 fluorophores. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which specific DNA was detected.
4. If the *Ct* value determined for the test sample is not greater than the boundary value, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check the correctness of selected threshold line level. If the result has been obtained with the correct level of threshold line (base line), the amplification and detection should be repeated for this sample.
5. If the *Ct* value determined for the test sample is not greater than the boundary value in two or more channels for the FAM, JOE, ROX and Cy5 fluorophores. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples.
6. If the *Ct* value determined for the test sample is less than the boundary value in the channel for the Cy5.5 fluorophore and the *Ct* value is less than the boundary value in one or more other detection channels (FAM and/or JOE, and/or ROX, and/or Cy5). The final fluorescence level in this/these channels is significantly lower than the final fluorescence level for C+ Het. There may be no mutation detectable in this channel. The analysis (beginning with the amplification stage) should be repeated for the sample at the optimal concentration (see *8.2. Preparing PCR*).

11 TRANSPORTATION

eSens BRCA1 SNP PCR kit should be transported at 2–8 °C for no longer than 5 days. PCR kit can be transported at 2–25 °C for no longer than 3 days.

12 STABILITY AND STORAGE

All components of the **eSens BRCA1 SNP PCR kit** are to be stored at 2–8 °C when not in use (except for PCR-mix-FL BRCA1, PCR-buffer-C and polymerase (TaqF)).

All components of the **eSens BRCA1 SNP PCR kit** are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-mix-FL BRCA1, PCR-buffer-C and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C

NOTE: PCR-mix-FL BRCA1 is to be kept away from light

13 SPECIFICATIONS

13.1 Analytical sensitivity (limit of detection)

Table 9

Test material	**The volume of sample for extraction, µl**	**Nucleic acid extraction kit**	**PCR kit**	**Analytical sensitivity (limit of detection), copies/ml**
Venous whole blood	100	RIBO-prep	ES4200B	$1x10^4$

13.2 Analytical specificity

The analytical specificity of **eSens BRCA1 SNP PCR kit** is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The PCR kit detects the presence of 5382insC (rs80357906), 4153delA (rs80357711), 300T>G (rs28897672) and 185delAG (rs80357914) mutations in the *BRCA1* gene. The analytical specificity was proved when testing 249 human genomic DNA samples with known genotypes for these mutations determined by sequencing with PyroMark Q24 pyrosequencer.

The nonspecific responses and incorrect mutation detection were not observed while testing the samples.

The clinical specificity of **eSens BRCA1 SNP PCR kit** was confirmed in laboratory clinical trials.

The information about interfering substances is specified in the *Interfering substances and limitations of using test material samples*.

13.3 Repeatability and reproducibility

Repeatability and reproducibility were determined by testing of positive and negative model samples. Quality control samples (QCS) were used for testing in concentration of $1x10^4$ copies/ml containing mutations in the *BRCA1* gene as positive sample and not containing mutations in the *BRCA1* gene (wild type) as negative sample. Each sample was tested in 10 repeats.

Repeatability conditions included testing in the same laboratory, by the same operator, using the same equipment within a short period of time. Reproducibility conditions included testing different lots of reagent kit in two independent laboratories, by different operators, using different equipment. The results are presented in Table 10.

Table 10

Mutation	Sample type	Repeatability		Reproducibility	
		Number of samples	Agreement of results, %	Number of samples	Agreement of results, %
5382insC	Positive	10	100	40	100
	Negative	10	100	40	100
4153delA	Positive	10	100	40	100
	Negative	10	100	40	100
300T>G	Positive	10	100	40	100
	Negative	10	100	40	100
185delAG	Positive	10	100	40	100
	Negative	10	100	40	100

13.4 Diagnostic characteristics

Table 11

The results of testing eSens BRCA1 SNP PCR kit in comparison with the reference assay

Mutation	The results of application of eSens BRCA1 SNP PCR kit		Results of using the reference assay	
			Positive	Negative
5382insC (rs80357906)	249 samples were tested	**Positive**	24	0
		Negative	0	225
4153delA (rs80357711)	249 samples were tested	**Positive**	3	0
		Negative	0	246
300T>G (rs28897672)	249 samples were tested	**Positive**	15	0
		Negative	0	234
185delAG (rs80357914)	249 samples were tested	**Positive**	4	0
		Negative	0	245

The diagnostic characteristics of the PCR kit were determined on 249 human genomic DNA samples.

Reagent kit for detection of genetic polymorphisms by pyrosequence using PyroMark series genetic analysis system was used as the comparison kit. The divergence between two assays was not observed while testing. The results are presented in Table 12.

Table 12

Diagnostic characteristics of eSens BRCA1 SNP PCR kit

Mutation	Diagnostic sensitivity*, (with a confidence level of 95 %), %	Diagnostic specificity**, (with a confidence level of 95 %), %
5382insC (rs80357906)	88-100	99-100
4153delA (rs80357711)	37-100	99-100
300T>G (rs28897672)	82-100	99-100
185delAG (rs80357914)	47-100	99-100

***** Relative sensitivity in comparison with applied reference assay.

****** Relative specificity in comparison with applied reference assay

14 QUALITY CONTROL

The production process, including batch release, is carried out in accordance with an established quality management system certified according to ISO 13485.

15 KEY TO SYMBOLS USED

	Catalogue number		Caution
	Batch code		Contains sufficient for <n> tests
	*In vitro* diagnostic medical device		Use-by Date
	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	**C+WT**, **C+ Het**	Positive controls of amplification
	Date of manufacture	**C-**	Negative control of extraction
	Authorized representative in the European Community	**NCA**	Negative control of amplification
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

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