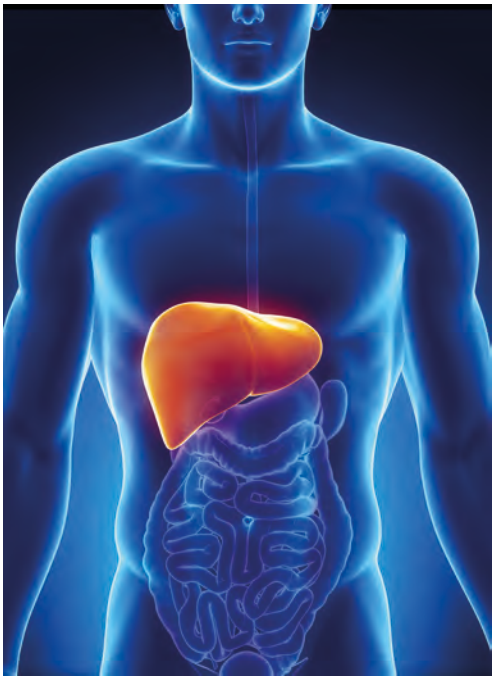
MutaCHIP® TOXO

DNA Macroarray Kit for the Examination of Mutations Associated with Detoxification



IVD

For in vitro diagnostics only

CE

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Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
www.immundiagnostik.com Tel.: +49 6251 70190-0
info@immundiagnostik.com Fax: +49 6251 70190-363

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1 Intended Use

The Toxo test kit is a biomolecular test for the detection of variations in genes, which protect the body from side effects and intoxications, caused by drugs or foreign substances.
Genomic DNA is used for the analysis by means of a DNA macroarray. The following mutations and polymorphisms are analysed:

Gene	Variation	rsNumber
COMT	Val158Met	rs4680
CYBA	242C>T	rs4673
CYP1A1	*2A (3798T>C)	rs4646903
CYP1A2	*1C (-3860G>A)	rs2069514
	*1F (-163C>A)	rs762551
CYP2B6	516G>T	rs3745274
	785A>G	rs2279343
CYP2C8	*3 (30411A>G)	rs10509681
CYP2C9	*2 (430C>T)	rs1799853
	*3 (1075A>C)	rs1057910
CYP2C19	*2 (681G>A)	rs4244285
	*3 (636G>A)	rs4986893
	*17 (-806C>T)	rs12248560
CYP3A4	*1B (-392A>G)	rs2740574
	*22 (15389C>T)	rs35599367
CYP3A5	*2 (27289C>A)	rs28365083
	*3 (6986A>G)	rs776746
Faktor II	20210G>A	rs1799963
Faktor V	1691G>A	rs6025
GSTM1	14kb Deletion	---
GSTP1	Ile105Val	rs1695
	Ala114Val	rs1138272
GSTT1	50kb Deletion	---
MDR1	3435C>T	rs1045642
MTHFR	677C>T	rs1801133
NAT2	191G>A	rs1801279
	341T>C	rs1801280
	481C>T	rs1799929
	590G>A	rs1799930
	857G>A	rs1799931
SOD2	Val16Ala	rs4880
VKORC1	*2 (-1639G>A)	rs9923231

2 Concept of the Assay

The target gene sequences are amplified by PCR. After a denaturation step, the amplification products are transferred to the Array Tube. Here the amplified products bind to the complementary probes immobilized on the Array. A washing step removes unspecifically bound fragments. In the next step the Conjugation Mix is added and binds to the probe-PCR-fragment complexes. Another washing step removes the unbound Conjugation Mix residues. Subsequent addition of the substrate results in a precipitation reaction only at those spots where the target gene sequence has previously bound. The overall precipitation pattern is detected by the image reader and interpreted by the corresponding software.

3 Kit Components

Toxo Kit		Volume		
Box	**Reagent**	**10-rxn**	**20-rxn**	**100-rxn**
1	PCR Mix A **(green)**	271 µL	520 µL	5 x 520 µL
	PCR Mix B **(yellow)**	271 µL	520 µL	5 x 520 µL
	PCR Mix C **(red)**	271 µL	520 µL	5 x 520 µL
	PCR Mix D **(blue)**	271 µL	520 µL	5 x 520 µL
	PCR Mix E **(white)**	271 µL	520 µL	5 x 520 µL
	PCR Mix F **(orange)**	271 µL	520 µL	5 x 520 µL
	Hybridisation Buffer **(transparent)**	1400 µL	2 x 1400 µL	11.5 mL
	Washing Buffer 1 **(blue dot)**	6 mL	12 mL	60 mL
	Washing Buffer 2 **(orange dot)**	7 mL	14 mL	2 x 35 mL
	Conjugation Mix **(black)**	1150 µL	2 x 1150 µL	11.5 mL
	Substrate **(brown, blue)**	1150 µL	2 x 1150 µL	11.5 mL
2	Polymerase **(purple)**	16 µL	31 µL	155 µL
	PC DNA **(brown)**	65 µL	65 µL	150 µL
	Array Tubes	10	20	100

4 Required Materials

Required materials - have to be ordered separately:

- Notebook + analysing software
- Imagereader
- Thermocycler (Peqlab Primus 25 advanced or Analytik Jena Biometra TAdvanced 96 [Aluminiumblock])
- Thermomixer with cooling function (BIOER Mixing Block MB-102)

The CE conformity is only given when the above mentioned components are used.

Required Materials - not provided:

- Pipettes:
  - 0.1 - 2.5 µL
  - 0.5 - 10 µL
  - 10 - 200 µL
  - 100 - 1000 µL
- 200 µL PCR tubes (sterile)

5 Storage and Shelf Life

- The light protection bag includes 5 Array Tubes with opened lid and has to be stored at room temperature.
  - An unsealed bag with remaining Array Tubes can be closed loosely (no tape).
  - Do not close the lids of the remaining Array Tubes.
  - Store the bag at a dark and dry place.
  - The Array Tubes in an opened bag are stable for several weeks under these conditions. We advise to use the remaining Array Tubes within four weeks to avoid even a minimal loss of performance.
- The polymerase and PC DNA (positive control DNA) are stored at -20 °C.
- All other components are stored at +2 to +8 °C.
- The substrate has to be protected from direct exposure to light.
- All reagents should stay at their indicated storage temperature until immediate use.

6 Working Conditions

The regulations and principles for working in a biomolecular laboratory have to be strictly followed.

- All steps have to be performed in an uninterrupted manner.
- All PCR reagents have to be kept cool during use.

7 Considerations and Precautions

- Use freshly extracted genomic DNA from EDTA whole blood.
  - The test was validated with the QIAamp DNA Blood Mini Kit.
- The Array Tubes ...
  - are for single-use only.
  - are only for *in vitro* diagnostics.
  - may not to be touched from below to prevent impurities on the lower side of the array.
  - may not run dry during the work flow.
  - have to be protected from sunlight and dirt.
  - have to be opened with two hands. Thereby no pressure should be exerted on the Array Tube.
  - are not allowed to be centrifuged.
  - may only be used with the herein mentioned reagents.
- The upper side of the array may not be touched with the pipette tip.
- Do not mix any reagents of different lots.

8 Sample Preparation

The template for PCR amplification is genomic DNA from EDTA whole blood. The DNA concentration should be between 5 and 40 ng/µL. The DNA purity ($OD_{260/280}$) should be between 1.8 and 2.0.

For the assay only high molecular (freshly extracted) DNA may be used.

9 Test Procedure

9.1 PCR Preparation

For the amplification of the target DNA six separate PCR reactions are required. All components have to be gently mixed before use (do not vortex the polymerase!) and shortly spun down. These are pipetted as described in the following schemes:

PCR reaction 1:

Reagent	Volume per 25 µL reaction
DNA (min. 20 ng - max. 160 ng)	4.0 µL
PCR Mix A **(green)**	20.8 µL
Polymerase **(purple)**	0.2 µL

PCR reaction 2:

Reagent	Volume per 25 µL reaction
DNA (min. 20 ng - max. 160 ng)	4.0 µL
PCR Mix B **(yellow)**	20.8 µL
Polymerase **(purple)**	0.2 µL

PCR reaction 3:

Reagent	Volume per 25 µL reaction
DNA (min. 20 ng - max. 160 ng)	4.0 µL
PCR Mix C **(red)**	20.8 µL
Polymerase **(purple)**	0.2 µL

PCR reaction 4:

Reagent	Volume per 25 µL reaction
DNA (min. 20 ng - max. 160 ng)	4.0 µL
PCR Mix D **(blue)**	20.8 µL
Polymerase **(purple)**	0.2 µL

PCR reaction 5:

Reagent	Volume per 25 µL reaction
DNA (min. 20 ng - max. 160 ng)	4.0 µL
PCR Mix E **(white)**	20.8 µL
Polymerase **(purple)**	0.2 µL

PCR reaction 6:

Reagent	Volume per 25 µL reaction
DNA (min. 20 ng - max. 160 ng)	4. µL
PCR Mix F **(orange)**	20.8 µL
Polymerase **(purple)**	0.2 µL

The PCR reactions have to be carefully mixed through and spun down. Subsequently place them into the thermocycler and use the PCR protocol described in 9.2.

9.2 PCR Protocol

Step	Temperature [°C]	Time [mm:ss]	Cycles
Lid Heat	99	---	---
Initial Denaturation	94	02:00	1 x
Denaturation	94	00:30	10 x
Primer Annealing	72	00:30	
Elongation	72	01:30	
Denaturation	94	00:30	25 x
Primer Annealing	60	00:30	
Elongation	72	01:30	
Final Elongation	72	05:00	1 x
Lid Heat	off	---	---
Storage	8	∞	1 x

After this step the PCR products can be stored at +2 to +8 °C up to 14 days. Do never store the PCR products below 0 °C.

If a thermocycler other than the in chapter 4 recommended ones is used, the PCR protocol has to be newly established (different thermocyclers have varying heating rates). Important: By doing so, the test loses its validity.

9.3 Array Tube Protocol

All reagents should stay at their indicated storage temperature until immediate use.
Please homogenize all reagents by inverting prior to use.

A) Preparation of the Hybridisation Buffer

If the Hybridisation Buffer is turbid or a precipitate is visible, the Buffer has to be heated at max. 60 °C for several minutes until it is clear (e.g. in the preheating thermoshaker). Subsequently, homogenize the buffer by inverting the tube. Let the Hybridisation Buffer cool down to room temperature before use.

B) Preparation of the Hybridisation Buffer

- Preheat the thermoshaker to **55 °C**.

C) Preparation of DNA samples

- Add **2 µL** of each PCR product (A, B, C, D, E and F) to a 200 µL PCR reaction tube, mix thoroughly and spin down.
- Denature the mixture at **95 °C** for **2 min** in the thermocycler
- Immediately add **100 µL** of Hybridisation Buffer to the denatured PCR product and mix by pipetting up and down.
- Transfer the mixture **completely** to the Array Tube, without touching the bottom of the Array.

D) Hybridisation

- Hybridise the Array Tube with the sample at **55 °C** and **550 rpm** for **30 min** in the thermoshaker.

E) Washing steps after hybridisation

- Take the Array Tube out of the thermoshaker. **The Hybridisation Buffer has to remain in the Array Tube until the target temperature of the next step is reached!**
- Set the thermoshaker to **52 °C**.
- When the target temperature is reached, completely remove the Hybridisation Buffer, also from the lid.
- Carefully add **500 µL** of **Washing Buffer 1** into the Array Tube.
- Incubate at **52 °C** and **550 rpm** for **5 min** in the thermoshaker.

F) Conjugation step

- Take the Array Tube out of the thermoshaker. **The Washing Buffer has to remain in the Array Tube until the target temperature of the next step is reached!**
- Set the thermoshaker to **21 °C**.
- Completely remove the Washing Buffer.
- Add **100 µL** of Conjugation Mix to the Array Tube.
- Incubate at **21 °C** and **550 rpm** for **15 min**.

G) Washing step after conjugation step

- Completely remove the Conjugation Mix.
- Carefully add of **500 µL Washing Buffer 2** to the Array Tube.
- Incubate at **21 °C** and **550 rpm** for **5 min**.

H) Precipitation

Caution: Do not shake the Array Tube during and after the precipitation reaction!

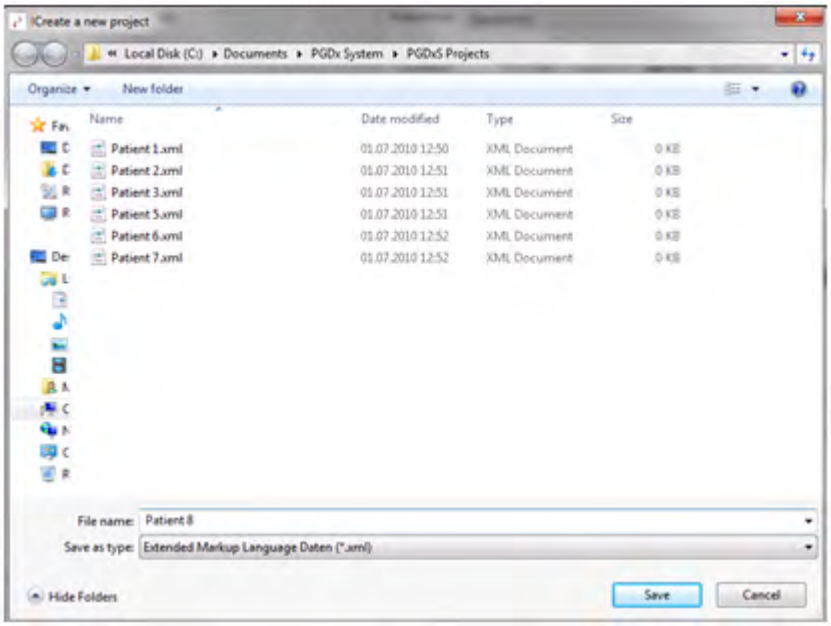
- Completely remove Washing Buffer 2.
- Add **100 µL** of Substrate to the Array Tube and incubate in the thermoshaker for **5 min** at **21 °C** **(Do not activate shaking function - use external timer)**.
- Thereafter, remove the Substrate completely and immediately add **100 µL** of **Washing Buffer 2**.
- Remove Washing Buffer 2 **completely** before performing the evaluation.
- Place the Array Tube into the image reader and proceed with the following chapter.

10 Evaluation

The evaluation is carried out with the provided genotyping software. The results are compiled into a report. For the evaluation of the Array follow the short instruction below.

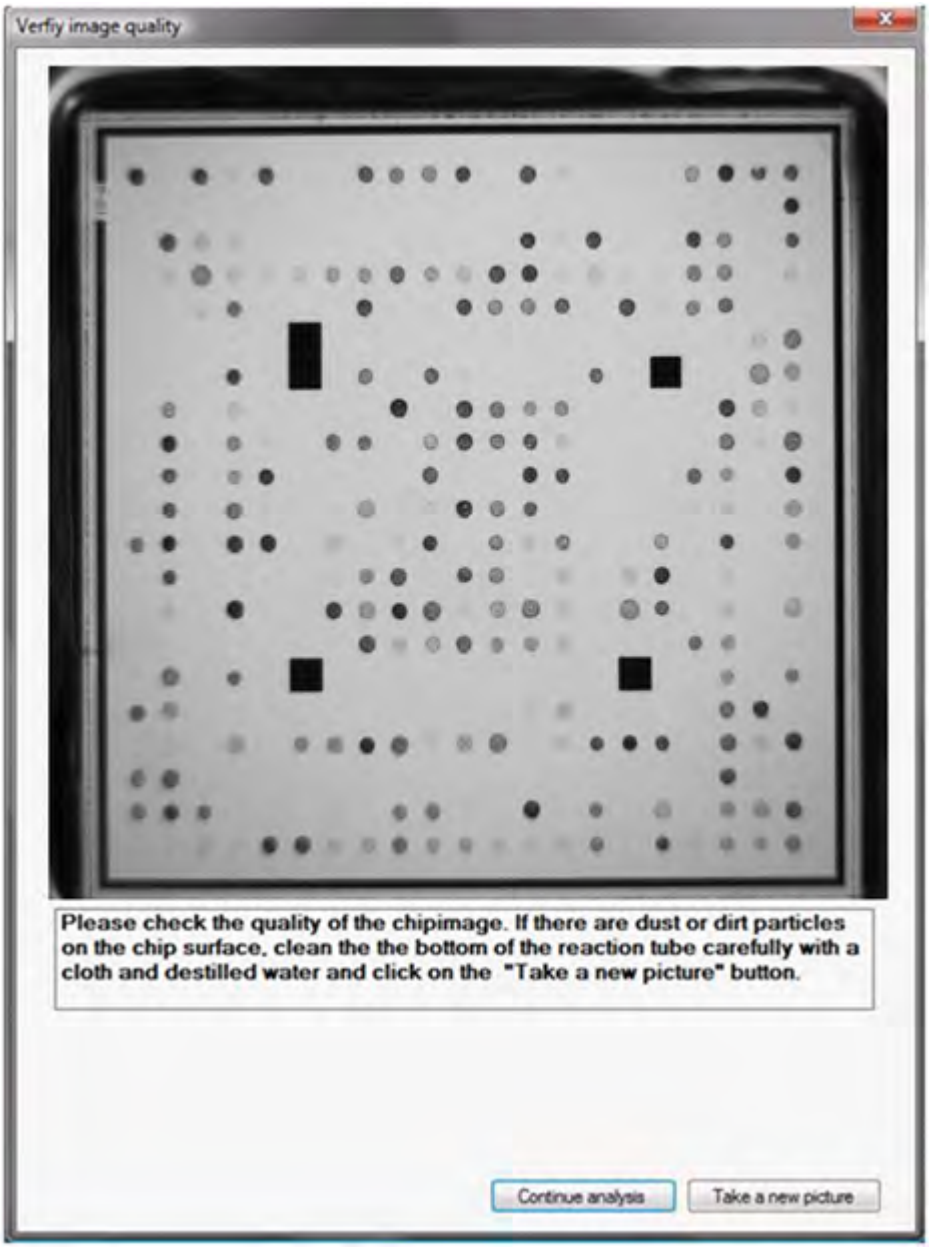
Step 1: Create a new project
Click on the button *Create a new project.*

Assign an arbitrary name for the experiment and subsequently save it by clicking on the button *Save*.



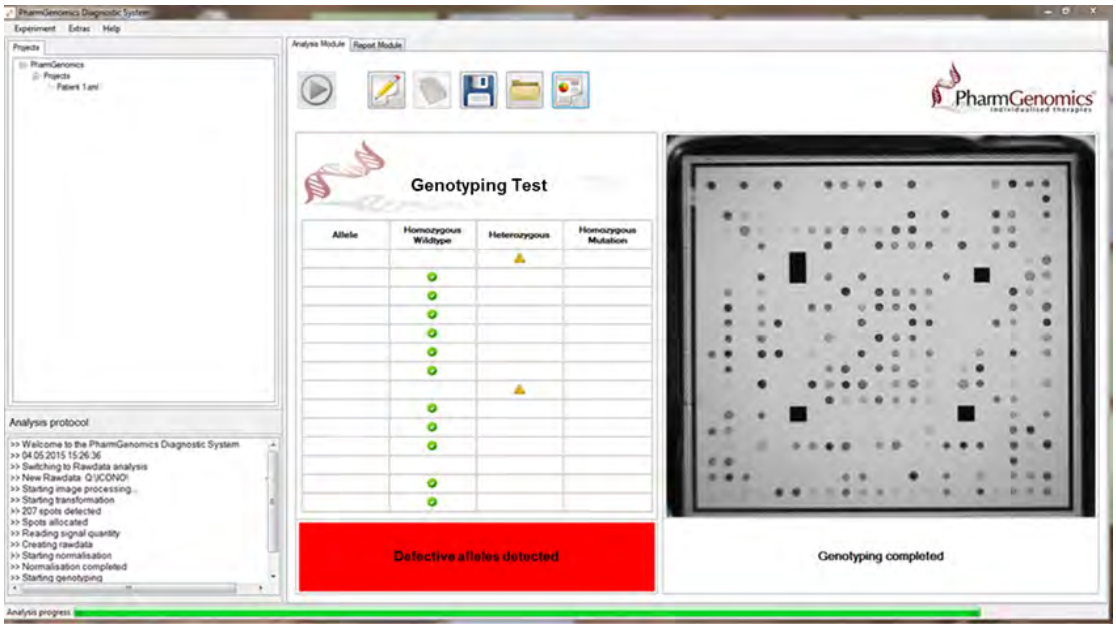
Step 2: Start the analysis program
Click on the button *Start the analysis program* to start the data evaluation.

Step 3: Quality check of the Array Tube
To ensure a correct analysis result, the picture quality of the Array Tube has to be checked. Dust particles on the bottom side of the Array Tube can interfere with the analysis. They can be removed with a soft and wet tissue. Click on the button *Continue Analysis* if the quality of the picture is comparable to the picture below. If not, click on the button *Take a new picture*. After cleaning the lower side of the Array Tube, restart the analysis program.



Step 4: Results

When the data analysis is completed, the results can be accessed in the analysis module/ genotyping module or in the diagnostic report.



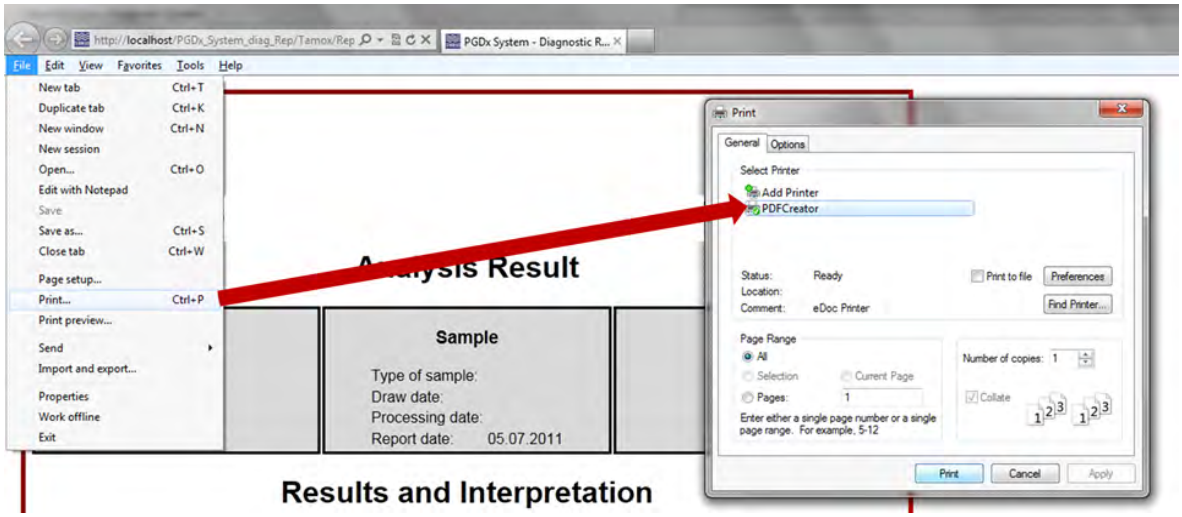
The used icons are explained in the table below.

	Homozygous wildtype: both alleles carry the wildtype variant
	Heterozygous mutated: one allele carries the wildtype variant, the other allele the mutated
	Homozygous mutated: both alleles carry the mutated variant
	The signal values of the probes for this genetic variation are too weak for a valid result. This could be a caused by a too weak amplification of the target sequence. The remaining signals of the assay are not influenced.
	Due to impurities no valid signal can be calculated for the variant. The remaining signals of the assay are not influenced.

Step 5: Diagnostic report

In the report module information regarding the patient and the attending physician can be entered. These will be transferred into the diagnostic report. To open the diagnostic report click on the button *Open diagnostic report*.

Additionally a .pdf document can be created and the report can directly be printed.



11 Result of the Positive Control

The following result is expected when the provided positive control DNA (PC DNA) is processed:

Gene	Variation	Result
COMT	Val158Met	Homozygous wildtype
CYBA	242C>T	Heterozygous mutation
CYP1A1	*2A (3798T>C)	Homozygous wildtype
CYP1A2	*1C (-3860G>A)	Homozygous wildtype
	*1F (-163C>A)	Homozygous mutation
CYP2B6	516G>T	Heterozygous mutation
	785A>G	Heterozygous mutation
CYP2C8	*3 (30411A>G)	Homozygous wildtype
CYP2C9	*2 (430C>T)	Homozygous wildtype
	*3 (1075A>C)	Heterozygous mutation
CYP2C19	*2 (681G>A)	Homozygous wildtype
	*3 (636G>A)	Homozygous wildtype
	*17 (-806C>T)	Heterozygous mutation
CYP3A4	*1B (-392A>G)	Homozygous wildtype
	*22 (15389C>T)	Heterozygous mutation
CYP3A5	*2 (27289C>A)	Homozygous wildtype
	*3 (6986A>G)	Homozygous mutation
Faktor II	20210G>A	Homozygous wildtype
Faktor V	1691G>A	Homozygous wildtype
GSTM1	14kb Deletion	At least one wildtype allele detected
GSTP1	Ile105Val	Heterozygous mutation
	Ala114Val	Homozygous wildtype
GSTT1	50kb Deletion	Homozygous wildtype
MDR1	3435C>T	Heterozygous mutation
MTHFR	677C>T	Heterozygous mutation
NAT2	191G>A	Homozygous wildtype
	341T>C	Heterozygous mutation
	481C>T	Heterozygous mutation
	590G>A	Homozygous wildtype
	857G>A	Homozygous wildtype
SOD2	Val16Ala	Homozygous wildtype
VKORC1	*2 (-1639G>A)	Homozygous wildtype

12 Troubleshooting

Problem	Solution
Running out of reagents.	All reagents are provided in a larger volume than needed to compensate for pipetting inaccuracies. Please contact the customer service.
Hybridisation Buffer is turbid / precipitates have formed.	Incubate the Buffer at max. 60 °C for several minutes until it is clear (e.g. in the preheating thermoshaker). Homogenize the buffers by inverting the tube.
The Washing Buffer 2 is turbid / precipitates have formed.	Please contact the customer service.
Deviations from the given protocol.	Any deviations from the given processing protocol can result in a loss of validity of the test. In this case the assay has to be repeated.
Poor picture quality: dust or similar residues visible on the array picture.	If the residues appear blurred, the bottom of the Array Tube needs to be cleaned. Wipe off impurities with one-directional movements using a soft, lintless cloth moistened with alcohol or disinfectant. If the residues appear sharp, the impurities are on the upper side of the array. To remove these, carefully add 100 µL of Washing Buffer 2 to the Array Tube and take it off immediately. Repeat this step if necessary.
Software Message: Warning! The signals of the biotin reference markers are too low. This could be a sign of failed or missed conjugation steps. Alternatively the enzyme could be degraded. The system will be stopped.	Were all Buffers removed completely during the Array processing? Check the Conjugation Mix and the Substrate for correct storage and shelf life. If this corresponds to the requirements, repeat the Array protocol. If not, please contact the customer service.
Software Message: Mix _ : Warning! The signals of the DNA probes indicated a failed amplification. The analysis cannot be continued.	The signal of the internal amplification control is too weak. This means, that either no amplification took place (e.g. due to insufficient DNA concentration; low polymerase activity as a result of damage during heavy mixing; PCR reaction was not mixed properly etc.) or the PCR mix was not added to the Array Tube. Repeat the assay.
Poor picture quality: dust or similar residues visible on the array picture.	Carefully clean the camera with a tissue or a cotton swab.
Software Message: No error description Error Code – 3011.	The reader is not connected correctly. Press “esc” for 3 seconds and subsequently connect the reader again.

Problem	Solution
Software Message: Warning! The signals of the DNA probes indicated a failed amplification.	Does the DNA concentration correspond to the requirements? If not, please repeat the extraction and prepare the PCR reaction again. Have you mixed the polymerase before use and the PCR reactions after setting them up? Repeat the PCR.
Software Message: Warning! This sample could not be analysed, please repeat the experiment. If the problem persists, please contact the PharmGenomics customer service.	A possible reason could be an invalid result. Please contact the customer service.
Software Message: Warning! Image analysis could not be started because too many probes could not be detected correctly. Maybe, there are some dirt particles on the lower side of the reaction tube, which interfere with the analysis. Please start the experiment again and take a new clear picture of the chip surface.	See poor picture quality. If the error continues to occur, contact the customer service.

13 Test Limitations

The accuracy of genetic testing is never 100%. However, an accuracy of more than 98% was determined based on the validation data. The attending physician is free to use the test results as a guidance in the decision making process in terms of diagnosis and therapy. However, these recommendations are based on genetic test outcomes and need to be interpreted in the context of medical history and known familiar risks of each individual patient. The attending physician is fully responsible for the final diagnosis and treatment.

The test only analyzes a selection of markers. For the detection of alleles the examined polymorphisms are indicated. Other rare alleles might be present, which are not covered by this method. Thus a negative test result does not exclude a risk for the patient in any form.