 <p>Niederhöchstädter Str. 62 D-61476 Kronberg / Taunus Tel. +49-6173-607930</p>	<p>Instructions for Use</p>	<p>CE</p>
<p>Version 2.7</p>	<p>RBC-FluoGene</p>	<p>QMS 11.17</p>

RBC-FluoGene

Intended Use:

Test kits for further analysis of pre-typed Blood Group samples
by End-Point Fluorescence Detection based on SSP PCR as well as real-time PCR

IVD

Article No.	Article	Reactions/ Test	Tests/ Plate	Tests/ Kit
001 086 040	RBC-FluoGene ABO basic	17	4	40
001 086 010	RBC-FluoGene ABO basic	17	1	10
001 081 030	RBC-FluoGene vERYfy	32	3	30
001 081 010	RBC-FluoGene vERYfy	32	1	10
001 091 101*	RBC-FluoGene vERYfy eXtend	96	1	10
001 082 040	RBC-FluoGene CDE	24	4	40
001 082 010	RBC-FluoGene CDE	24	1	10
001 083 040*	RBC-FluoGene D weak/variant	16	4	40
001 083 010*	RBC-FluoGene D weak/variant	16	1	10
001 085 010	RBC-FluoGene Rare	32	1	10
001 087 048	RBC-FluoGene D-Screen	1	12	48
001 088 048	RBC-FluoGene Vel-Screen	2	12	48
001 089 010	RBC-FluoGene KKD	16	1	10
001 090 010	RBC-FluoGene MNS	15	1	10

* The purchase price of this product includes limited, non-transferable rights under European Patent EP 1 047 777 B1

CHANGES TO VERSION 2.6:

- changes cover page, header:
Version 2.1 & QMS 11.17
- General revision due to market launch of the FluoQube System

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1 GENERAL PRODUCT INFORMATION

1.1 Principle of the Method

inno-train's molecular FluoGene detection system is based on PCR SSP (SSP: "Sequence Specific Priming"). The Polymerase chain reaction (PCR) enables amplification of defined DNA sequences [1]. After successful amplification, the DNA target sequence from genomic DNA is present in detectable concentration. SSP is a variant of PCR in which only the sequences of the primers are responsible for a specification of alleles to be detected [2]. For complete PCR SSP analysis therefore, several amplifications are performed in parallel. PCR samples in which the primer binds to its specific target have a specific amplificate following PCR, while samples without this primer specific binding do not. Primers are also present for amplification of an internal control (gene sequence of the human growth hormone, HGH). If no specific product is present after PCR, the amplificate of this positive control must be clearly detectable. Detection of the PCR products is performed by measuring fluorescence signals and not by gel electrophoresis as in conventional PCR systems.

Each PCR test contains at least two fluorochromes coupled to oligonucleotide probes which can be differentiated in the emission spectrum of the fluorochrome. At least one probe is specific for the target sequence and one for the internal HGH control sequence.

Fluorescence detection can be performed as an endpoint method in the fluorescence reader (**inno-train** FluoVista) or in real time during the PCR run in the real-time PCR cyclor (**inno-train** FluoQube).

Endpoint detection detects the emissions of the various fluorochromes in the FluoVista before and after PCR and calculates the difference using the FluoGene analysis software.

In the FluoQube, the increase in emissions of the various fluorochromes during the PCR run is detected. The Q values and the CT values (cycle threshold, beginning of the exponential growth of an amplification curve) are determined from these fluorescence values. These are evaluated as positive or negative using the FluoGene evaluation software. The increase in fluorescence and Q values above specific thresholds reflects a positive amplification.

1.2 RBC-FluoGene Test Systems

inno-train's RBC-FluoGene test systems enable molecular typing of various blood groups. Different oligonucleotide mixes that are pre-aliquoted and dried in PCR trays enable the amplification of genetic markers. Each of these oligonucleotide mixes contains primers and probes labelled with different fluorescence dyes:

- A RBC specific probe (fluorochrome 1)
- A HGH specific probe for amplification control (fluorochrome 2)
- Possibly an additional RBC specific probe in case of multiplex reactions (fluorochrome 3)

The position of the negative/contamination control containing a HGH specific oligonucleotide mix is marked on the tray by a black line.

1.2.1 RBC-FluoGene ABO basic

Oligosaccharides are responsible for the antigenic characteristics of the erythrocyte ABO receptors. These carbohydrates are not coded by DNA sequences but are synthesized by enzymes. The sequence of carbohydrate specific glycosyl transferases can in turn be identified through PCR. In the ABO blood group system the transferases transfer the terminal sugar residues of the carbohydrate chains from glycoproteins and glycolipids to the fucose of the erythrocyte membrane so that A antigens (N-acetyl-galactosamine) and/or B antigens (galactose) are produced.

The DNA sequences responsible for the expression of the different glycosyl transferases are located on chromosome 9 (9q34).

The RBC-FluoGene ABO basic system is capable of distinguishing the most frequent antigens A, A₂, B, O₁ and O₂.

1.2.2 RBC-FluoGene vERYfy

This kit is suitable for typing various human red cell blood group alleles. Serological ambiguous Ds, unclear blood groups within polytransfused patients or patients generating allo- or auto-antibodies and/or showing a positive DAT can be screened and identified with the RBC-FluoGene vERYfy kit. In the Rh system exons 3, 5, 10 and psi (a 37 bp insertion in intron 3) of the *RHD* gene and the C, c, E, e and C^w alleles are detected. Furthermore typing of the blood groups Kell (KEL1/KEL2), Kidd (JK1/JK2), Duffy with the alleles FY1(A), FY2(B), FYX and FYnull (GATA-box mutation), MNS with the alleles MNS1(M), MNS2(N), MNS3(S), MNS4(s), U+var(P2) and U+var(NY), as well as Dombrock (DO1/DO2) are covered with this kit.

1.2.3 RBC-FluoGene CDE

Two genes located on chromosome 1 in region 1p34 to 1p36, the *RHD* gene and the *RHCE* gene, are responsible for the expression of the CDE rhesus antigens. Both genes consist of ten exons. While the term Rhesus little d expresses the absence of the complete *RHD* gene and thus of the D receptor, the CcEe antigens are characterized by sequence differences in the *RHCE* gene. In addition, the CDE test system detects the *RHCE* SNPs 733C>G and 1006G>T. Predictions on the deviated RH:10/RH:20 (V/VS) phenotypes can be made with these specificities. For detailed information about the *RHCE* variants containing these SNPs please refer to the accompanying worksheet of the kit. For reasons of clarity the software indicates only a positive detection of the SNPs. Besides the D positive and D negative phenotypes, expression of so-called D variants occurs in rare cases (0.2 - 1% in Europeans). A distinction is made between the partial D types (e.g. D categories) and the weak D types.

This RBC-FluoGene CDE system enables a "normal" D type to be distinguished from the various partial D types by molecular detection of the different exons of the *RHD* gene.

So-called hybrids are generated by exchanging one or more exons of the *RHD* gene for the corresponding exons of the *RHCE* gene. The most well-known of these hybrids are detected with the present system.

In addition, the system allows detection of the C, c, E, e and C^w antigens.

As required by international specialist associations, the allelic variants of *RHD* psi and d(C)ces are also detected with the FluoGene CDE system. More detailed information about the resolution of the system is given on the respective specificity tables.

1.2.4 RBC-FluoGene D weak / variant

The weak D antigens are alleles of the *RHD* gene on chromosome 1 and do not differ in their immunogenic protein structure (extracellular) from the D antigen. The molecular characteristic of the weak D types are various point mutations, which lead to amino acid exchanges in the intracellular or transmembrane region of the respective antigen. All weak D antigens have in common a lower receptor density on the cell surface compared to D positive cells. The resulting lower concentration of Rh/D epitopes on the erythrocyte membrane depends on the weak D type present and can lead to very strong weakening of agglutination in the classical haemagglutination test. Using the RBC-FluoGene D weak/variant makes it possible to detect the most frequent weak D alleles (type 1, 1.1, 2, 3, 4, 4.0, 4.1, 4.2 (DAR), 5, 11, 14, 15, 17) clearly. At least 95% of all observed weak D phenotypes can be classified as weak D alleles type 1 to type 5. Furthermore the three most common DEL alleles DEL(M295I), DEL(K409K) and DEL(IVS3+1G>A) are determined.

1.2.5 RBC-FluoGene Rare

With this test system the rare blood group alleles Diego (DI1/DI2), Wright (DI3/DI4), Cartwright (YT1/YT2), Lutheran (LU1/LU2), Kell (KEL3/KEL4 and KEL6/KEL7), Colton (CO1/CO2) and Knops (KN1/KN2) can be identified. These blood group systems are biallelic with one frequent and one rare allele.

1.2.6 RBC-FluoGene D-Screen

The test system enables the detection of *RHD* exons 3, 5 and 10 in one single reaction. Thereby exon 10 is detected by color 1, while the exons 3 and 5 are detected together by color 2. The internal HGH control is labelled with color 3. For example, the screening test is suitable for a molecular confirmation of serological D negative samples. Positive screening reactions should be further investigated.

1.2.7 RBC-FluoGene Vel-Screen

The Vel blood group was first described in 1952, but until 2013 the molecular background remained obscure. Homozygosity for a 17 nucleotide deletion in the third exon of the *SMIM1* gene on chromosome 1p36 causes the rare Vel negative phenotype.

1.2.8 RBC-FluoGene KKD

The **Kell** system is based on a gene complex located on chromosome 7 with at least 3 gene loci. On locus 1 K+k- (KEL*01.01) und k+ (KEL*02) are present in turn; on locus 2 Kp(a+) (KEL*02.03) and Kp(b+) are located; Js(a+) (KEL*02.06) and Js(b+) are present on locus 3.

Distinguishing between KEL*01.01 / KEL*02, known as K (Kell) and k (Cellano) in serological nomenclature, is based on an amino acid exchange of threonine by methionine in exon 6 of the gene.

With the RBC-FluoGene KKD kit KEL*01.01 and KEL*02 and the alleles of the Kp and Js loci are detected.

Important note: The specificities Kp(a+), Kp(b+), Js(a+) and Js(b+) have been implemented in RBC-FluoGene KKD from lot R989077S. The required pipetting volumes have not been changed compared to previous lots.

The **Kidd** system consists of a total of three different antigens, Jka (JK*01), Jkb (JK*02) and Jk- (JKnull). JK*01 and JK*02 differ by one sequence difference, which leads to an amino acid exchange at position 280 of the antigen. Both SNPs are detected with the RBC-FluoGene KKD kit.

The **Duffy** system (*DARC* gene) comprises the FY*01, FY*02, FY*X and FY*null (GATA) alleles to be detected by the FluoGene system which are located reciprocally at the gene locus. In serological nomenclature, the FY*01 allele corresponds to the Fy^a antigen and the FY*02 allele corresponds to the Fy^b antigen. The weakly expressed FY*X allele is detected serologically as Fy^bweak (=Fy^X).

In contrast to serological methods, the KKD SSP enables a clear detection of the immunologically relevant antigen Fy^bweak (FY*X). The FY*null (Fy-) allele, which is present in the frequency of approx. 1.4% of the Central European population, does not produce a Fy receptor. Since *Plasmodium vivax* requires the Duffy receptor for malaria infection, those FY*null alleles represent a selection advantage, causing an increase of its incidence to up to 68% in regions with a high malaria risk (e.g. Africa). The Duffy system also acts as a chemokine receptor.

1.2.9 RBC-FluoGene MNS

The RBC-FluoGene MNS test system detects the alleles GYPA*01 (GYPA*M) and GYPA*02 (GYPA*N) (corresponds to the phenotypes MNS:1 (or M+) and MNS:2 (or N+), as well as GYPB*03 (GYPB*S) and

GYPB*04 (GYPB*s) (corresponds to the phenotypes MNS:3 (or S+) and MNS:4 (or s+)). Furthermore, the alleles U+var(P2) (GYPB*P2/GYP*He(P2)), U+var(P3) (GYPB*04N.01) and U+var(NY) (GYPB*NY/GYP*He(NY)) are detected with the MNS typing kit.

Important note: The specificity U+var(P3) has been implemented in the RBC-FluoGene MNS kit from lot R990087S. The required pipetting volumes have been changed compared to previous lots. For details please see the table on page 7.

1.2.10 RBC-FluoGene vERYfy eXtend

RBC-FluoGene vERYfy eXtend was developed for extended blood group typing and represents a combination of **inno-train's** blood group typing kits. Samples can be characterized for the blood group systems RHD/RHCE, KEL, JK, FY, MNS, DO, LU, YT, DI, VEL, CO and KN with only one single test. For further information please refer to the accompanied specificity table of the kit.

2 MATERIAL

2.1 Components of the RBC-FluoGene Kits

- **White, barcoded 96-well PCR trays with labelled cover foil**

Contain the dyed and dried oligonucleotide mixes consisting of blood group sequence specific and internal control primers as well as fluorescence labelled oligonucleotide probes. One typing comprises a defined number of reactions (see table 1).

Table 1: Kit specific components

Kit	Typings/ Kit	PCRs/ Typing	No. PCR Trays	No. FluoMix tubes	No. optical foils
ABO basic	40	17	10	40	10
ABO basic	10	17	10	10	10
vERYfy	30	32	10	30	10
vERYfy	10	32	10	10	10
CDE	40	24	10	40	10
CDE	10	24	10	10	10
D weak/variant	40	16	10	40	10
D weak/variant	10	16	10	10	10
Rare	10	32	10	10	10
D-Screen	48	1	4	4	4
Vel-Screen	48	2	4	4	4
KKD (from lot R989077S)	10	16	10	10	10
MNS (from lot R990087S)	10	15	10	10	10
vERYfy eXtend	10	96	10	10	10

- **Reaction tubes with FluoMix**

One tube is sufficient for one typing.

FluoMix contains dNTPs, PCR buffer and *Taq* Polymerase.

- **Optical foils for sealing**

- **Instructions for Use**

- **Kit/Lot No. documentation**

Quality certificate

Specificity table

Information sheet with changes to the previous lot

- **Versioned Lot files Update CD containing:**

Kit and lot specific files and allele databases for import into the FluoGene Software with instructions

2.2 Storage and Shelf-Life

RBC-FluoGene kits are dispatched chilled. After receipt the kits should be stored at -20°C to -30°C . The shelf-life of the individual components and the entire system are stated on the respective label.

2.3 Additionally Required Materials

For Amplification	For Detection and Evaluation
<ul style="list-style-type: none"> • Distilled water (without fluorescence) e.g. “LiChrosolv” (Merck) • Sample DNA (Ratio $A_{260/280}$: $1.8 \pm 10\%$) • Pipettes (1 – 1000μl) • Pipette tips with filters • Dispenser with fixed volume (15 μl) • Thermocycler for endpoint detection (see 3.4.2) • FluoPad, compression pad for PCR (inno-train, Art. No.: 002 07C P01) • FluoApp, applicator for application of the optical foil (inno-train, Art. No.: 002 07F A01) 	<ul style="list-style-type: none"> • inno-train FluoVista Analyzer (Art. No.: 006 010 001) or • inno-train FluoQube Real-Time-PCR Instrument (Art. No.: 005 010 001) • FluoGene Software

3 PROCEDURE

3.1 Obtaining Samples

EDTA or citrate blood should be used as starting material for the procedure since heparin residues following DNA extraction can inhibit the PCR. In DNA extraction from blood, the leukocytes serve as DNA source, but in general genomic DNA of any origin can be used for the method (hair, buccal swab, etc.). The concentration of the sample DNA has to be determined prior to test setup. The DNA should be dissolved and diluted in distilled water (without fluorescence) in a concentration of approx. 1 ng/ μl . Alternatively the DNA can be mixed undiluted with the FluoMix and be adjusted with H₂O to a final concentration of 0.5 ng/ μl . The purity ratio $\text{OD}_{260}/\text{OD}_{280}$ should be $1.8 \pm 10\%$.

3.2 Precautions

Due to the closed RBC-FluoGene system there is no risk of contamination as no PCR product can leak out after the PCR preparation. According to existing regulations we recommend the following safety measures:

To avoid contamination within the PCR method:

- Spatial separation of the pre-PCR area (DNA isolation, storage, PCR sample) from the post-PCR area (thermocycler, fluorescence reader)
- Components of the post-PCR area must not get into the pre-PCR area.
- Use pipette tips with aerosol protection in the pre-PCR area.

To perform molecular methods in Blood Group typing:

- Experience in Blood group typing and DNA diagnostics is necessary.

3.3 Create a Job in the FluoGene Software

For the FluoGene PCR trays and DNA samples to be tested a job has to be created in the evaluation software according to the FluoGene Software Manual. The FluoGene Software automatically generates a PCR protocol as a guideline for the PCR setup of the test.

3.4 PCR / Amplification

The procedure is the same for all RBC-FluoGene kits, except of RBC-FluoGene D-Screen and RBC-FluoGene Vel-Screen (see 3.4.1):

- ⇒ Thaw PCR trays, FluoMix and DNA samples.
- ⇒ The negative/contamination control is in the black marked well.
- ⇒ The FluoMix is ready-to-use and aliquoted for one typing per tube. Before adding DNA, vortex FluoMix thoroughly and pipette 7.5 μl into the negative control well.
- ⇒ Add 7.5 μl of distilled water into the negative control well.

⇒ Afterwards add the diluted DNA (conc. 1 ng/μl ± 50%) to the remaining FluoMix (according to table), again vortex thoroughly and distribute 15 μl aliquots to the remaining tubes of the typing (preferably with a hand dispenser at the top edge of the tube walls).

As an alternative you can also directly pipette the DNA into the FluoMix tube and dilute to the final volume with water. The required final DNA amounts and the final end volume are stated in the table below.

For a better visibility the already pipetted reactions show a slight coloring (the contamination control is reddish, the specific reactions blueish).

Kit	Volume FluoMix	Volume DNA sample (1 ng/μl ± 50%) / Test	DNA amount (± 50%)	Final volume FluoMix, H ₂ O and DNA
ABO basic, KKD, D weak/variant, MNS (from lot R990087S)	150 μl	150 μl	150 ng	300 μl
CDE	210 μl	210 μl	210 ng	420 μl
vERYfy, Rare	270 μl	270 μl	270 ng	540 μl
MNS (until lot R990076S)	90 μl	90 μl	90 ng	180 μl
vERYfy eXtend	780 μl	780 μl	780 ng	1560 μl

⇒ Cover the PCR tray with the adhesive optical foil and close by pressing firmly (use disposable lab gloves and an applicator, e.g. FluoApp by **inno-train**). Avoid finger marks and dirtying the foil.

⇒ Gently tap the PCR tray on the work surface so that all the liquid is at the bottom of the reaction tube.

Optional: centrifuge the PCR tray shortly

⇒ Avoid liquid splashes and condensate on the foil during the whole procedure. If necessary transfer the PCR tray to thermocycler and incubate for approx. 30 sec. with heated lid.

⇒ **For using the FluoVista Analyzer: The pre-read has to be performed within 15 minutes after PCR setup.** Transfer the PCR tray to the FluoVista Analyzer and perform the pre-read. Please note that the correct orientation of the PCR tray is provided by metal bolts on top of the FluoVista Carrier Plate. It is automatically pulled into the device when orientation is correct (jagged side to the right) and must not be pushed or pulled out by force.

⇒ Transfer the PCR tray to thermocycler or FluoQube, cover with a clean PCR pressure pad, e.g. FluoPad by **inno-train**, make sure the heated lid is clean and start PCR program.

3.4.1 PCR setup D-Screen and Vel-Screen

The D-Screen and Vel-Screen test systems consist of 12 typings per PCR tray. It does not contain a negative control. If you like to perform a negative control you can use any of the 12 reactions. Just pipette 7.5 μl FluoMix and 7.5 μl H₂O directly into the PCR tray. The reactions are located in the rows B1-B6 (D-Screen) and B1-B6 (Vel+), C1-C6 (Vel-) and F1-F6 (Vel+) and G1-G6 (Vel-).

D-Screen:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	1	3	5	7	9	11						
C												
D												
E												
F	2	4	6	8	10	12						
G												
H												

Vel-Screen:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	1+	3+	5+	7+	9+	11+						
C	1-	3-	5-	7-	9-	11-						
D												
E												
F	2+	4+	6+	8+	10+	12+						
G	2-	4-	6-	8-	10-	12-						
H												

Note: the software assigns the tray column by column from top down. Therefore the DNA-FluoMix solutions have to be applied accordingly from top down and from left to right (see numbers in graph).

One FluoMix tube (120 µl volume D-Screen and 230 µl volume Vel-Screen) is sufficient for one PCR tray with 12 typings.

Kit	Volume FluoMix incl. dead volume	Volume DNA sample (1 ng/µl ± 50%) / Test incl. dead volume	DNA amount (± 50%) in 15 µl finale volume	Final volume FluoMix, H ₂ O and DNA
D-Screen	9 µl	9 µl	7.5 ng	15 µl
Vel-Screen	17 µl	17 µl	7,5 ng	2x 15 µl

DNA and FluoMix have to be mixed thoroughly before adding to the PCR tray.

Alternatively: 7.5 µl FluoMix and 7.5 µl DNA (1 ng/µl) can be directly pipetted into the well if the sealed PCR tray is shortly centrifuged afterwards.

3.4.2 PCR Program (for use with FluoVista Analyzer)

1x Initial	40x Cycles	Cooling Down	
95°C 2 min.	95°C 15 s 60°C 60 s	20°C 3 min	20°C max. 15 min.

Validated thermocyclers and notes:

Manufacturer	Model	Ramp Rate	Important Note
Applied Biosystems	GeneAmp PCR System 9700	Max.	Aluminium block not suitable
Applied Biosystems	Veriti	9700 Mode	
Analytik Jena	Biometra TProfessional 96	2.5°C	
Analytik Jena	Biometra TAdvanced	2.5°C	Denaturation temperature must be increased to 96°C in all steps.

The use of other thermocyclers has to be validated by the user.

3.4.3 PCR Program (for use with FluoQube Real-Time Instrument)

All settings for PCR and parameters for measurement are already preset and are used automatically for FluoGene from a secured template.

1x Initial	38x Cycles
96°C 2 min.	95°C 15 s 60°C 49 s (+read*)

*The read is performed during the 60°C step. The following color channels must be selected: “Blue”, “Green” and “Orange”. The ramp rate for PCR must be adjusted to 2.5 °C/s.

The use of the qTOWER³ (Analytic Jena) is possible under certain conditions. To do this, prior to using the FluoGene method a service visit from **inno-train** must take place in order to make certain settings on the device and to install the corresponding software to be acquired. For further information please ask your local inno-train representative.

3.5 Fluorescence Detection

- ⇒ The endpoint fluorescence detection takes place in the **inno-train** FluoVista Analyzer (post-read). Please make sure that steaming up of the optical foil by condensation is avoided (see above) and reading is performed max. 15 min. after thermocycling.
- ⇒ The fluorescence detection of the **inno-train** FluoQube Real-Time Instrument is carried out directly during the PCR run.

3.6 Calculation of the Data

- ⇒ Is carried out automatically by the FluoGene Software.

4 EVALUATION

The evaluation can be carried out after endpoint fluorescence detection (pre- and post-read) or after real-time PCR.

For the endpoint detection with the FluoVista, the background fluorescence of the different fluorochromes measured during the pre-read before PCR is automatically subtracted from the fluorescence values of the post-read by the FluoGene Software.

In the FluoQube, the increase in emissions of the various fluorochromes during the PCR run is detected. The Q values and the CT values (cycle threshold, beginning of the exponential growth of an amplification curve) are determined from these fluorescence values.

These calculated values are evaluated as positive or negative using the FluoGene evaluation software. The increase in the fluorescence or the Q values above specific thresholds reflects thereby a positive amplification. If the respective value is below the threshold value, the reaction is evaluated as negative.

In positive RBC specific reactions the internal control reaction may be suppressed because of competitive primer effects. In the event that the specific reaction is negative and the internal control drops out, the reaction would not be considered for the result and marked with a red question mark.

Further information is provided in the FluoGene Software manual.

5 PERFORMANCE DATA / PERFORMANCE ASSESSMENT

As part of performance assessment studies, all RBC-FluoGene kits were tested with pre typed (molecular biological) samples. In all kits the results agreed to 100%.

In quality controls each oligonucleotide mix is tested for correct positivity and negativity.

A safe typing result is obtained if 7.5 ng of DNA are used per well. The composition of the oligonucleotide mixes allows reliable identification of the blood group alleles listed in the specificity tables. The accuracy and reproducibility of the reactivity of the individual oligonucleotide mixes were checked using control samples with known alleles.

The quality of the results that are achieved with the RBC-FluoGene kits depends largely on the reagents and materials used. For this reason it is recommended that the tests are performed using the additionally required materials described in chapter 2.3. Any deviations from the procedure (e.g. use of alternative thermocycler) must be validated by the user.

6 GENERAL WARNINGS AND PRECAUTIONS

- ⇒ In samples of human origin there is still a potential risk of infection even after DNA extraction. Therefore gloves and lab coats should be worn when performing the FluoGene method and all recommendations on handling infectious material should be followed.
- ⇒ Inadequate patient material may affect the results of analysis.
- ⇒ The fluorophores are photosensitive. Therefore the FluoGene plates should be processed quickly.
- ⇒ Reagents should not be used after their expiry date.
- ⇒ Only use optical foils delivered with the kits for sealing the FluoGene plates.
- ⇒ Only use the **inno-train** FluoVista Analyzer and the **inno-train** FluoQube Real-Time PCR Instrument for measuring fluorescence.
- ⇒ Reagents must be used lot specific.
- ⇒ The specifications given in these Instructions for Use for the FluoGene workflow have to be strictly followed. Deviations may lead to false positive or false negative reactions of the primer-/probe mixes and therefore cause incorrect typing results.







Further information and telephone assistance at: +49 6173-607930, or by email to support@inno-train.de







7 TROUBLE SHOOTING

Problem	Possible cause	Solution
Failure of reactions / false negative reactions	Aliquoted volume is too high	Check pipette setting
	Impure DNA quality	Measure OD ₂₆₀ / OD ₂₈₀ . A quotient of 1.8 is optimal.
	Magnetic beads within PCR setup	Separate beads by magnet or centrifugation
	PCR inhibitors in distilled water	Only use fresh distilled water
	Low DNA concentration	Measure DNA concentration and adjust to 1 ng/μl. For DNA dilution only use fluorescence-free water.
	Mastermix and DNA were not mixed well	Thoroughly vortex mastermix-DNA solution
	Mastermix was not pipetted in well	Optical control. After adding the mastermix the solution is colored blue.
	Mastermix was pipetted with air bubbles into well	Optical control. When air bubbles are observed centrifuge the plate shortly or knock it carefully on the table.
	Incorrect amplification conditions	Check the thermocycler. See points 3.4.2 and 3.4.3 to see which thermocyclers have been validated for performing the method.
	Wells are not correctly sealed by optical foil	Make sure that the optical foil seals each well tightly. Use the FluoApp for this purpose. The usage of the FluoPad during PCR is recommended.
	Splashes or dirt on optical foil	Change optical foil
	Fogging or dirty optical foil	Incubate the PCR tray in thermocycler with running lid heating for app. 30 sec. or replace the optical foil. Touch the foil only with clean disposable gloves. Make sure that the FluoVista is not placed directly under the flow of the air condition.
False positive reactions	Plate is not fixed correctly on FluoVista Carrier Plate during post-read	Check the correct position of the plate prior to each read
	Period between PCR setup and pre-read is longer than 15 minutes and/or storage between PCR setup and pre-read or respectively between thermocycler and post-read in the light	Measure the plate within 15 minutes after PCR setup and always keep in the dark.
	Aliquoted volume is too low	Check pipette setting
	Aliquoted DNA concentration is too high	Measure DNA concentration and set to 1 ng/μl. Only dilute DNA with fluorescence free water.
	Incorrect amplification conditions	Check the thermocycler. See points 3.4.2 and 3.4.3 to see which thermocyclers have been validated for performing the method.
Red or yellow pens used on plate	Only use black or blue inks for writing on the plate.	
Air bubbles in well during pre-read	Destroy potential air bubbles by knocking off or centrifuging the plate.	
Evaporation due to untight foil sealing	Use FluoApp for tightly pressing the foil on the plate. Take care of tight sealing at the edges.	
Period between end of PCR and fluorescence detection is too long	Perform the post-read within 15 min after the end of thermocycling.	
Splashes or dirt on optical foil	Change optical foil	

Problem	Possible cause	Solution
False positive reactions	Fogging or dirty optical foil Period between PCR setup and pre-read is longer than 15 minutes	Incubate the PCR tray in thermocycler with running lid heating for app. 30 sec. or replace the optical foil. Touch the foil only with clean disposable gloves. Make sure that the FluoVista is not placed directly under the flow of the air condition. Measure plate within 15 minutes after the PCR setup

8 EXPLANATION OF SYMBOLS

-  Note accompanying documents
-  Lot number
-  Follow Instructions for Use
-  Contents sufficient for <n> tests
-  Observe upper temperature limit
-  FluoMix

-  Expiry date
-  Article number
-  Manufacturer
-  In vitro diagnosticum
-  PCR tray
-  optical foil

9 LITERATURE

1. Mullis KB, Faloona F: Specific synthesis of DNA in vitro via polymerase catalysed chain reaction. Meth. Enzym. 1987;155:335-350
2. Newton CR, Graham A, Heptinstall E, Powell SJ, Summers C, Kalsheker N, Smith JC, Markham AF: Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). Nucleic Acids Research 1989;17:2503-2516.