en
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Version 2.6

HLA-FluoGene®

Important information:

HLA-FluoGene® kits with 15 µl (FluoMix tubes with colorless screw caps) and 8 µl (FluoMix tubes with orange screw caps) reaction volumes are currently available.

These instructions for use apply to the kits with 8 µl reaction volume.

Instructions for Use

RUO

For Research Use Only. Not for use in diagnostic procedures.

"Not FDA cleared device"

Article no.	Article	Wells/Test	Tests/plate	Tests/kit
002 085 010	HLA-FluoGene^{384} Match	192	1	10
002 085 020	HLA-FluoGene^{384} Match	192	2	20
002 082 010	HLA-FluoGene^{NX} ABCDRDQ	96	1	10
002 083 005	HLA-FluoGene^{NX} Match	192	1 test on 2 plates	5
002 074 010	HLA-FluoGene ABDR	96	1	10
002 070 010	HLA-FluoGene A	24	1	10
002 071 020	HLA-FluoGene B	48	2	20
002 071 010	HLA-FluoGene B	48	1	10
002 072 010	HLA-FluoGene C	24	1	10
002 073 010	HLA-FluoGene ABC	96	1	10
002 081 010	HLA-FluoGene DRDQDP plus	80	1	10
002 077 010	HLA-FluoGene DRDQ	32	1	10
002 077 030	HLA-FluoGene DRDQ	32	3	30
002 084 020§	HLA-FluoGene DRDQ plus	40	2	20
002 079 010§	HLA-FluoGene DPB1	48	1	10

§ Upon request only

Versions 2.3 to 2.5 were created during the IVDR certification process. The last public version was version 2.2. Changes compared to version 2.2 (created on August 14, 2024):

- Cover page: Listing of HLA-FluoGene DPB1 as a product "available on request"
- Section 1.2 Intended use updated in accordance with IVDR requirements
- Addition to Section 1.4 Information on HLA-FluoGene^{NX} Match and HLA-FluoGene^{384} Match
- Note in Section 1.5 that no external controls are necessary.
- Update to Section 5.2 regarding sample requests
- Updating of clinical performance data in Section 7
- Change of storage temperature from -35°C to -20°C to the original storage temperature of at least -20°C
- Updating of Section 5.2 regarding the handling of DNA
- Chapter 9: Adding the symbol for single use only
- Addition to the literature (point 7 and 8)

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1. GENERAL PRODUCT INFORMATION

HLA typing is used to determine the histocompatibility of the donor and recipient, which is important for the success of allogeneic transplants.

1.1 Special note

Originally, a reaction volume of 15 µl was used to carry out HLA-FluoGene®. With the aim of accelerating the PCR, the reaction volume was reduced to 8 µl. Some kits newly developed in this format were given the label "NX" in the product name. The HLA-FluoGene® kits, which were later adapted to the 8 µl reaction volume, will not be renamed.

In the past, for example, the label "NX" was used for PiU profiles as an expression for the 8 µl reaction volume. It is therefore expressly stated here that 8 µl reaction volume and "NX format" are to be used as synonyms.

These instructions for use are valid for HLA-FluoGene® kits with 8 µl reaction volume, this includes the variants HLA-FluoGene^{384}, HLA-FluoGene^{NX} and HLA-FluoGene®. For the sake of simplicity, only HLA-FluoGene® is mentioned. Possible differences are explicitly mentioned.

1.2 Intended use

HLA-FluoGene® is intended for molecular genetic typing of HLA alleles of class I (A, B, C) and class II (DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1, DPB1) from DNA for the determination of histocompatibility in organ transplantations.

HLA-FluoGene® is performed semi-automatically or manually and provides qualitative results.

1.3 Requirements for users

HLA-FluoGene® should only be used by trained personnel experienced in molecular genetic techniques. Transplantation guidelines and EFI / DGI standards as well as other national laws and guidelines in the respective country must be observed, especially in case of doubtful typing results.

1.4 Test principle

The test principle is based on the sequence-specific primer polymerase chain reaction (SSP-PCR), which enables the amplification of defined DNA sequences [1]. The sequence of the primer at the 3'-end discriminates the different HLA alleles [2, 3, 4, 5]. For a complete SSP-PCR analysis, several amplifications are performed in parallel. Each well contains pre-aliquoted and dried primer-probe mixes for the amplification of specific HLA target sequences as well as an internal control sequence (fragment of the *HGH* gene, HGH = human growth hormone). If no specific product is present after PCR, the amplicon of this internal control must be clearly detectable.

The typing result is derived from the pattern of the various positive reactions.

To improve the resolution of the different kits, it is possible to detect different alleles in one well as so-called multiplex mixes. Which wells contain multiplex mixes and which wells contain so-called singleplex mixes (i.e. in addition to the HLA-specific sequence, only the sequence for the internal control is detected) can be found in the batch-specific specificity table.

The detection of PCR products is based on the TaqMan® Probe principle using hydrolysis probes (=TaqMan® probes) to which various fluorescent dyes are coupled. Up to 4 different fluorescent colors are used in the multiplex mixes. The amplicons are differentiated by the respective fluorescence color. Which alleles are detected with which fluorescence color can be found in the batch-specific specificity table.

In the case of amplification, the probe is attached to the amplicon and the 5' - 3' exonuclease activity of the polymerase contained in the test system enables the degradation of the bound probes, resulting in a fluorescent signal [6].

Fluorescence detection can be performed as an endpoint method in inno-train's fluorescence reader "FluoVista" or in real time during the PCR run in inno-train's real-time PCR device "FluoQube" (applies to all products except HLA-FluoGene^{384} Match).

For HLA-FluoGene^{384} Match, fluorescence detection is performed in real time during the PCR run in the "FluoQube^{384} " real-time PCR device from inno-train.

HLA-FluoGene^{NX} Match and HLA-FluoGene^{384} Match are intended for molecular genetic typing of HLA alleles of class I (A, B, C) and class II (DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1, DPB1) with a resolution comparable to single antigen antibody tests from DNA for the determination of histocompatibility in organ transplantations.

The evaluation of HLA-FluoGene® is automated by the FluoGene software.

1.5 Description of the controls

HLA-FluoGene® does not require calibration or external controls.

Each well contains primers for the amplification of an internal control (fragment of the *HGH gene*).

The negative control (contamination control, NC) is pre-dropped in a separate well - the position can be found in the corresponding specificity table and is also displayed on the pipetting scheme in the FluoGene software. The negative control can be visually identified by the red coloration of the oligonucleotide mix.

1.6 Description of the test reagents

The pre-dropped oligonucleotide mixes contain HLA-specific and internal control primers (fragment of the *HGH gene*) as well as fluorescence-labeled oligonucleotide probes. The oligonucleotide mixes of the specific reaction have a bluish color and the negative control (contamination control) has a reddish color.

The FluoMix contains dNTPs, PCR buffer and *Taq polymerase*.

2. MATERIALS

2.1 Components of the kit

All HLA-FluoGene® kits contain PCR plates (96 well); HLA-FluoGene^{384} Match contains 384 well PCR plates with a variable number of wells containing the pre-dripped oligonucleotide mixes, FluoMix and PCR foils.

The FluoMix of the HLA-FluoGene® kits with 8 µl reaction volume ("NX format") has an orange lid for better differentiation from the kits with 15 µl reaction volume.

This does not apply to HLA-FluoGene^{384} Match, because only this product contains the FluoMix in a 5 mL container.

Details can be found in the table below:

Article no.	Product	Tests/ Kit	Wells/ Test	Included components
002 085 010	HLA-FluoGene^{384} Match	10	192	10x PCR plate 10x FluoMix 10x optical foil
002 085 020	HLA-FluoGene^{384} Match	20	192	10x PCR plate 10x FluoMix 10x optical foil
002 082 010	HLA-FluoGene^{NX} ABCDRDQ	10	96	10x PCR plate 10x FluoMix 10x optical foil
002 083 005	HLA-FluoGene^{NX} Match	5	192	10x PCR plate (5x plate A, 5x plate B) 10x FluoMix

Article no.	Product	Tests/ Kit	Wells/ Test	Included components
				10x optical foil
002 074 010	HLA-FluoGene ABDR	10	96	10x PCR plate 10x FluoMix 10x optical foil
002 070 010	HLA-FluoGene A	10	24	10x PCR plate 10x FluoMix 10x optical foil
002 071 020	HLA-FluoGene B	20	48	10x PCR plate 20x FluoMix 10x optical foil
002 071 010	HLA-FluoGene B	10	48	10x PCR plate 10x FluoMix 10x optical foil
002 072 010	HLA-FluoGene C	10	24	10x PCR plate 10x FluoMix 10x optical foil
002 073 010	HLA-FluoGene ABC	10	96	10x PCR plate 10x FluoMix 10x optical foil
002 081 010	HLA-FluoGene DRDQDP plus	10	80	10x PCR plate 10x FluoMix 10x optical foil
002 077 010	HLA-FluoGene DRDQ	10	32	10x PCR plate 10x FluoMix 10x optical foil
002 077 030	HLA-FluoGene DRDQ	30	32	10x PCR plate 30x FluoMix 10x optical foil
002 084 020	HLA-FluoGene DRDQ plus	20	40	10x PCR plate 20x FluoMix 10x optical foil
002 079 010	HLA-FluoGene DPB1	10	48	10x PCR plate 10x FluoMix 10x optical foil

The following product-related documents are available for download on the inno-train website:

- Instructions for use
- Specificity table
- Certificate
- List of CWD/CIWD alleles (common, intermediate and well-documented alleles)
- Information sheet with a description of all changes compared to the previous batch
- Kit files and allele databases for import into the FluoGene software

2.2 Additional materials required

Reagents	Provider
Aqua dest. without inherent fluorescence	N/A.

2.3 Required accessories and devices

Instruments	Provider
DNA extraction system	N/A.
Pipettes (0.5 - 1000 µl)	N/A.
Pipette tips with filter	N/A.
Thermal cycler (only when used with FluoVista):	See chapter 6.3
• GeneAmp® PCR System 9700	Applied Biosystems
• Veriti	Applied Biosystems
• TProfessional 96	Biometra
• TAdvanced	Biometra
• VeritiPro	Applied Biosystems
• SimpliAmp	Applied Biosystems
Pressure mat (not for HLA-FluoGene^{384} Match)	e.g. inno-train, Art. No.: 002 07C P01
Applicator for pressing on the optical film	e.g. inno-train, art. no.: 002 07F A01
Plate centrifuge	N/A.
FluoVista	inno-train, Art. No.: 006 010 001
Alternative to FluoVista - Real-Time devices:	
• FluoQube (for all except HLA-FluoGene^{384} Match)	inno-train, Art. No.: 005 010 001
• FluoQube^{384} (only for HLA-FluoGene^{384} Match)	inno-train, Art. No.: 008 010 000
FluoGene evaluation software	inno-train, Art. No.: 006 010 003S
Additionally for HLA-FluoGene^{384} Match:	
• 16-channel pipette	e.g. integra VIAFLO 16-Ch 5 - 125 µl; Article no. 4642
• 10 mL Reservoir Base	integra Art. No.: 4306
• 10 mL Disposable Reservoir, sterile, polystyrene	integra Art. No.: 4332

3. STORAGE AND SHELF LIFE

3.1 Storage

HLA-FluoGene® kits are shipped refrigerated. The storage temperature is -20°C or below. The products have a shelf life of two years; the expiry dates of the individual components and the overall system are indicated on the respective label.

Remove the optical foils from the kit and store at room temperature.

3.2 Stability after the first opening

FluoMix and the PCR plates containing primer-probe mix are intended for single use only and are discarded after use. The shelf life stated on the label applies to the control DNAs.

3.3 Sterile product

HLA-FluoGene® is not sterile and does not need to be sterilized.

4. SAFETY INSTRUCTIONS, PRECAUTIONARY MEASURES AND DISPOSAL INSTRUCTIONS

4.1 Safety instructions and precautionary measures

- To avoid contamination within the PCR method
  - Spatial separation of the pre-PCR area (DNA isolation, storage, PCR preparation) from the post-PCR area (thermal cycler, fluorescence detection).
  - Components of the post-PCR area must not enter the pre-PCR area.
  - Use of DNase/ RNase-free pipette tips with aerosol protection in the pre-PCR area.
- The pre-dried PCR plates are intended for single use only.
- The reagents must not be used after the expiry date.
- Only reagents packaged together (PCR plate and FluoMix) may be used together. FluoMix from a different batch must not be used.

4.2 Limits of the procedure

With HLA-FluoGene®, only selected DNA sequence segments are examined. Unknown mutations in the primer or probe binding region can lead to the primer-probe mix in question failing and providing no or an incorrect typing result.

4.3 Warnings about potentially infectious material

HLA-FluoGene® does not contain any potentially infectious material. The sample material is extracted DNA.

Blood is used as the starting material for DNA extraction. There is always a potential risk of infection with samples of human origin. Therefore, gloves should be worn during DNA extraction and the performance of HLA-FluoGene® and all recommendations for handling infectious material should be followed.

4.4 Interfering substances

Purified DNA is required for typing with HLA-FluoGene®. EDTA or citrated blood should be used as starting material for DNA extraction, as heparin residues can inhibit PCR.

When extracting with magnetic beads, make sure that all bead residues are removed, as the beads can inhibit the PCR.

4.5 Disposal of the product

When disposing of samples, unused reagents and waste, the local and country-specific waste regulations must be observed.

The Material Safety Data Sheet (MSDS), which is available in the download area of the inno-train website, must also be observed.

Biological material should be inactivated before disposal. Disposable items should be autoclaved or incinerated after use.

Spills of potentially infectious material should be removed immediately with absorbent paper towel and the contaminated areas wiped with a suitable standard disinfectant or 70% alcohol.

Materials used to clean up spills, including gloves, should be inactivated before disposal.

5. ADDITIONAL REQUIREMENTS

5.1 Requirements for the facility, requirements for the qualification of users

HLA-FluoGene® can be used in any molecular biology laboratory.

The product may only be used by qualified personnel with experience in molecular genetic techniques and HLA typing.

5.2 Requirements for the sample treatment

The starting material for DNA extraction is fresh or frozen whole blood samples. Alternatively, leukocyte suspensions (buffy coat), but also genomic DNA of any origin (hair, cheek swab, etc.) can be used.

Sampling, sample processing, storage, and transport must be carried out in accordance with the instructions provided with the DNA extraction kit used for biological material. The user is responsible for ensuring that the requirements for sample handling are met in accordance with the regulatory and official requirements of the country in which they are carried out.

The requirements for DNA for HLA-FluoGene® are set out below:

The concentration of the sample DNA must be determined before starting the test. The sample DNA should be dissolved and diluted in distilled water (without inherent fluorescence) alternatively in TE buffer (10 mM Tris, pH 7-8, max. 1 mM EDTA) at a concentration of 1 ng/µl ± %. Alternatively, the DNA can also be added undiluted to the FluoMix and adjusted to a final concentration of 0.5ng/µl with H_2O. The purity quotient OD260/OD280 must be 1.8 ± 10%.

If the DNA is not used immediately for testing, it can be stored for 2 months at 2–8°C or 2 years at -20°C [7]. Longer storage times must be validated by the user.

Frozen DNA can be frozen and thawed up to 10 times [8].

5.3 Preparation

A new job must be created for the HLA-FluoGene® PCR plates and DNA samples to be tested in accordance with the FluoGene software instructions for use. The FluoGene software automatically generates a PCR protocol as a working template with all the necessary information.

Thaw the FluoMix and DNA sample before use and mix well. Also bring the plate to room temperature.

5.4 Installation qualification

The performance of HLA-FluoGene® requires fluorescence measurement by the FluoVista analyzer or real-time detection by the FluoQube. When commissioning a new FluoQube, FluoQube^{384}, or FluoVista system, an IQOQ (installation and functional qualification) must be carried out by inno-train service personnel. To ensure the best possible quality and fault-free use of the FluoGene application, FluoVista, FluoQube and FluoQube^{384} must be checked once a year by inno-train service personnel.

A FluoGene Color Control Test (order number 006 010 00S, or order number 005 07CO 010 for FluoGene Color Control 384) is available for regular checking of the FluoVista or FluoQube and FluoQube^{384} by the user. These tests are available to check the measurement accuracy by measuring 3 different dyes at opposite positions on the plate. The FluoVista or FluoQube and FluoQube^{384} should be checked with the Color Control Test once a week if the device is used daily or at least once a month.

When using the FluoVista analyzer, a thermal cycler is also required. The installation, qualification and maintenance of these devices are the responsibility of the user. In the course of FluoVista maintenance, the thermal cycler is also checked with a FluoGene Cycler Control Test and approved for the FluoGene application.

5.5 Recommendations for quality control

The EFI and ASHI guidelines recommend regular participation in external proficiency tests. For internal quality control of new batches, it is recommended to use your own reference DNA samples or e.g. the inno-train Reference DNA Panel (Art. No.: 001 K00 004).

5.6 Metrological traceability

A new batch of HLA-FluoGene® and HLA-FluoGene^{NX} is released if the specified number of quality control tests yield clearly positive and clearly negative results, i.e. the measured fluorescence values are within the specified measuring ranges.

The deviations of a new batch of HLA-FluoGene® compared to the previous batch are described on the batch-specific "Information Sheet".

6. TEST PERFORMANCE

6.1 PCR set-up HLA-FluoGene® and HLA-FluoGene^{NX}

- Thaw all reagents completely before use, vortex the FluoMix well and centrifuge briefly.
- The FluoMix is ready to use and aliquoted for typing. Pipette 4 µl into the negative control tube before adding the DNA.
- Add 4 µl distilled water to the negative control vessel.
- Then add diluted DNA (conc. 1 ng/µl ± 50%) to the remaining FluoMix (see table for volume), vortex again thoroughly and distribute in 8 µl aliquots to the remaining tubes of the typing (preferably with a hand dispenser on the upper edge of the tube walls).

  Alternatively, the amount of DNA specified in the table can be added directly to the FluoMix and filled up to the corresponding final volume with H2O.

  Note: If the DNA was extracted using magnetic beads, this step must prevent any remaining beads from entering the reaction mix. The magnetic beads can be separated by a 1-2-minute centrifugation step at 1000 rcf or with the aid of a magnetic stand.

Kit	FluoMix volume	Sample DNA volume (1 ng/µl ± 50%) / test	DNA quantity (± 50%)	Final volume FluoMix, H_2O and DNA
HLA-FluoGene^{NX} ABCDRDQ HLA-FluoGene^{NX} Match HLA-FluoGene ABDR	430 µl	430 µl	430 ng	860 µl
HLA-FluoGene A HLA-FluoGene C	120 µl	120 µl	120 ng	240 µl
HLA-FluoGene B HLA-FluoGene DPB1	240 µl	240 µl	240 ng	480 µl
HLA-FluoGene ABC	430 µl	430 µl	430 ng	860 µl
HLA-FluoGene DRDQDP plus	360 µl	360 µl	360 ng	720 µl
HLA-FluoGene DRDQ	150 µl	150 µl	150 ng	300 µl
HLA-FluoGene DRDQ plus	190 µl	190 µl	190 ng	380 µl

For automatic PCR setup using the PiU1 pipetting unit, the required DNA volumes may deviate from this table. In this case, the volumes in the PiU1 pipetting profile apply.

- Cover the PCR plate with adhesive optical foil and seal it by pressing firmly and evenly (use disposable laboratory gloves and a pressure spatula, e.g. FluoApp from inno-train). Avoid fingerprints and soiling on the foil.
- **Centrifuge the PCR plate for 1 - 2 minutes at 1000 rcf**

- During the entire method procedure, liquid splashes and condensation on the foil must be avoided at all costs. If necessary, incubate the PCR plate for approx. 30 sec. in the thermal cycler with the lid heater switched on.
- **When using the FluoQube,** the plate is transferred to the FluoQube after centrifugation, paying attention to the correct orientation (barcode of the plate points to the right), place a clean pressure mat (e.g. FluoPad) on top and start the PCR (chapter 6.4).
- **When using the FluoVista** analyzer:
    - Perform the pre-read within 15 minutes after PCR preparation. Transfer the PCR plate to the FluoVista. The correct orientation of the PCR plate is determined by metal pins on the FluoVista carrier tray. Carefully insert the FluoVista carrier loaded with the PCR plate into the device in the correct orientation (serrated long side pointing to the right) until it is automatically drawn into the device. The carrier must not be pushed in or pulled out by force.
    - Transfer the PCR plate to the thermal cycler, cover with a clean pressure mat (e.g. FluoPad) and start the PCR (section 6.3).
    - After the end of the PCR, the post-read must be performed within 15 minutes. The plate can be measured directly after it has cooled down to 20°C for 3 minutes (penultimate step in the PCR program).

6.2 PCR set-up for HLA-FluoGene^{384} Match

- Thaw all reagents completely before use, vortex the FluoMix well and centrifuge briefly.
- The FluoMix is ready to use and aliquoted for typing. Pipette 4 µl into the negative control tube before adding the DNA.
- Add 4 µl distilled water to the negative control vessel.

  Then add diluted DNA (conc. 1 ng/µl ± 50%) to the remaining FluoMix (see table for volume), vortex thoroughly again and divide into 8 µl aliquots into the remaining typing tubes.

  Alternatively, the amount of DNA specified in the table can be added directly to the FluoMix and filled up to the corresponding final volume with H2O.

  Note: Foam formation due to vigorous shaking of the tube must be avoided.

  Note: If the DNA was extracted using magnetic beads, the remaining beads must be prevented from entering the reaction mix in this step. The magnetic beads can be separated by a 1–2-minute centrifugation step at 1000 rcf or with the aid of a magnetic stand.
- The easiest way to load the plate is to use the integra 16-channel pipette. It is advisable to set the dispensing speed as high as possible (setting 10). To do this, add the FluoMix DNA mixture to a suitable 10 mL reservoir.
  It is recommended to start loading in row 12 and to remove the pipette tip that would dip into the negative control when row 1 is reached in order to exclude the addition of DNA to the negative control.

  Alternatively, the plate can be loaded automatically using the Viaflo Assist. The corresponding pipetting profile can be provided. If you are interested, please contact support@inno-train.de.

Kit	FluoMix volume	Sample DNA volume (1 ng/µl ± 50%) / test	DNA quantity (± 50%)	Final volume FluoMix, H_2O and DNA
HLA-FluoGene^{384} Match	1050 µl	1050 µl	1050 ng	2100 µl

- Cover the PCR plate with adhesive optical foil and seal it by pressing firmly and evenly (use disposable laboratory gloves and a pressure spatula, e.g. FluoApp from inno-train). Avoid fingerprints and soiling on the foil.

- **Centrifuge the PCR plate for at least 5 minutes at 1000 rcf.**
- During the entire method procedure, liquid splashes and condensation on the foil must be avoided at all costs. If necessary, incubate the PCR plate for approx. 30 sec. in the thermal cycler with the lid heater switched on.
- Transfer the PCR plate to FluoQube^{384}, ensuring the correct orientation (barcode on the plate facing forwards), make sure the heated lid is clean and start the PCR (section 6.5).

6.3 PCR program for use with FluoVista

1x Initial		40x cycles		Cooling down	
96°C	2 min.	96°C	15 sec.	20 °C 3 min	20 °C max. 15 min
		60°C	30 sec.		
		68 °C	5 sec.		

The program was validated with the following thermal cyclers:

Manufacturer	Model	Heating rate	Important note
Applied Biosystems	GeneAmp PCR System 9700	Max.	Aluminum block not suitable
Applied Biosystems	Veriti	9700 mode	-
Analytics Jena	Biometra TProfessional 96	2,5 °C	-
Analytics Jena	Biometra TAdvanced	2,5 °C	-
Applied Biosystems	SimpliAmp	2,5 °C	-
Applied Biosystems	VeritiPro	2,5 °C	-

As part of the annual maintenance of the FluoVista, the thermal cycler is also checked by a cycler control test specially developed for FluoGene and approved for the FluoGene application after passing the test. The use of other thermal cyclers must be validated by the user. It is recommended to use the FluoGene Cycler Control test for this purpose.

6.4 PCR program for use with FluoQube

The settings of the PCR and reading parameters of the FluoQube are already preset on delivery and are automatically applied by a protected template "FluoGene NX.rts". As the abbreviation "NX" is synonymous with the 8 µl reaction volume, the name of the template will be changed to FluoGene-8.rts in the future.

1x Initial		38x cycles	
96 °C	2 min.	96 °C	15 sec.
		60 °C	18 sec.(+Read*)
		68 °C	5 sec.

***** The fluorescence measurement takes place during the 60°C step. The "Blue", "Green" and "Orange" channels must be selected. The FluoQube must be programmed with a heating rate of 2.5 °C/sec. When using the "FluoGene NX.rts" (later: "FluoGene-8.rts") template, these settings are already pre-installed.

The PCR program was validated with the FluoQube Real Time PCR device.

The use of the qTOWER³ (Analytik Jena) is possible under certain conditions. Before using the FluoGene method, a service visit from inno-train must take place in order to make certain settings on the device and install the software to be purchased accordingly. For more information, please call +49-6173-607930 or send an e-mail to support@inno-train.de.

6.5 PCR program for use with FluoQube^{384}

The settings of the PCR and reading parameters of the FluoQube^{384} are already preset on delivery and are automatically applied by a protected template "FluoGene 384.rts".

1x Initial		38x cycles	
96 °C	2 min.	96 °C	15 sec.
		60 °C	10 sec (+Read*)
		68 °C	5 sec.

***** The fluorescence measurement takes place during the 60°C step. The "Blue", "Green", "Orange" and "Red" channels must be selected. The FluoQube^{384} must be programmed with a heating rate of 2.5 °C/sec for the 96 °C and 68 °C steps and with a heating rate of 2.0 °C for the 60 °C step. When using the "FluoGene 384.rts" template, these settings are already pre-installed.

The PCR program was validated with the FluoQube^{384} real-time PCR device.

The use of the qTOWER^{384} (Analytik Jena) is possible under certain conditions. Before using the FluoGene method, a service visit from inno-train must take place in order to make certain settings on the device and install the software to be purchased accordingly. For more information, please call +49-6173-607930 or send an e-mail to support@inno-train.de.

6.6 Fluorescence detection

In real-time PCR using FluoQube or FluoQube^{384}, fluorescence is measured in real time during the PCR run. The measurement is performed in each cycle after annealing.

For endpoint fluorescence detection using the FluoVista analyzer, the background fluorescence is determined before the PCR run (pre-read) and then after the PCR run (post-read) when the amplificates formed have been replicated. Care must be taken to avoid fogging of the optical film due to condensation and any contamination of the film and the measurement must be carried out within 15 minutes of the end of the PCR program.

6.7 Evaluation

The evaluation is carried out using the FluoGene evaluation software. The respective device-specific raw data is automatically imported into the software and the typing result is calculated and displayed based on the fluorescence measurements. In the case of a positive specific reaction, the internal control reaction may fail due to competitive primer effects. However, if the control reaction fails in a reaction in which the specific reaction is also negative, this reaction is marked with a question mark in the FluoGene software.

FluoVista: During endpoint detection, the FluoGene software calculates the difference between the two measurements (pre- and post-read) for each position and each fluorochrome. The resulting delta values are evaluated by the software as positive or negative. The basis for the evaluation is a cut-off value stored in the kit and batch-specific kit file, which must at least be reached in order to evaluate the reaction as positive.

FluoQube and FluoQube^{384}: In real-time PCR, the FluoGene software calculates so-called Q values for each position and each fluorochrome from the fluorescence values, the amplification curve and CT values (cycle threshold, start of the exponential growth of an amplification curve). These are compared with the cut-off values stored in the kit and batch-specific kit file and a reaction is evaluated as positive (exceeding the cut-off) or negative (falling below the cut-off).

Further and more detailed information on the evaluation software can be found in the FluoGene software instructions for use.

7. PERFORMANCE CHARACTERISTICS

7.1 Analytical performance characteristics

7.1.1 Analytical specificity and cross-reactivity

In the course of developing the primer mixes, the primers to be used were evaluated with regard to their GC content and thus their melting behavior, their behavior to form hairpin structures and primer dimers, and subjected to an NCBI-BLAST (Basic Local Alignment Search Tool) analysis.

Possible cross-hybridization is prevented by pairing suitable primers and is regularly checked in the batch-related quality controls.

Cross-reactivities between alleles from different gene loci cannot be excluded and are mentioned in the batch-specific specificity table.

7.1.2 Limit of detection (Limit of Detection)

The recommended DNA concentration for carrying out HLA-FluoGene® is 1 ng/µl ± 50%. To determine the detection limit pre-typed DNA samples were tested in different dilutions. HLA-FluoGene^{NX} Match was used for the tests.
The detection limit was determined to 0.17 ng/µl DNA.

7.1.3 Reproducibility (intra-assay precision)

10 DNA samples were tested with HLA-FluoGene^{NX} Match on five non-consecutive days.

The intra-assay precision was determined to 99.3 %.

7.1.4 Repeatability (inter-assay precision)

10 DNA samples were tested with two batches of HLA-FluoGene^{NX} Match.

The inter assay precision was determined to 97%

7.2 Clinical performance characteristics

The correct reactivity was determined with 103 samples pre-typed with a CE-marked test system using HLA-FluoGene ABC and DRDQDP plus. The sensitivity (PPA) determined is listed in the table below.

	Number of samples	Sensitivity (PPA)
HLA-A	103	99.5 %
HLA-B	103	100%
HLA-C	103	100%
HLA-DRB1	103	100%
HLA-DRB3	103	100%
HLA-DRB4	103	100%
HLA-DRB5	103	100%
HLA-DQA1	103	100%
HLA-DQB1	103	100%
HLA-DPA1	103	100%
HLA-DPB1	103	99.1%

Tests on clinical performance characteristics with HLA-FluoGeneNX Match and HLA-FluoGene384 Match kits show comparable results to those shown in the table.

7.3 Additional information on the performance characteristics

The positive reactions form a pattern that can be assigned to specific alleles. HLA-FluoGene® products are tested every six months as part of an external proficiency testing program.

During each batch-related quality control, each primer-probe mix is checked for correct positivity and negativity.

8. GENERAL INFORMATION

8.1 Reporting of any serious incidents

Any serious incident that has occurred during the use of HLA-FluoGene® must be reported to inno-train: support@inno-train.de.

In the event of a serious risk to the patient, the competent authority of the Member State in which the user and/or patient is located must be informed.

8.2 IT security

The HLA-FluoGene® kits do not contain any programmable systems. Therefore, the requirements for IT security and IT network characteristics are not applicable. The FluoGene software required for evaluation fulfills the IT security requirements.

8.3 Summary of Safety & Performance (SSP)

The summary report required by IVDR 2017/746 can be found in the EUDAMED database.

9. EXPLANATION OF THE SYMBOLS

⚠	Observe accompanying documents	⌛	Can be used until
LOT	Batch designation	REF	Item number
📖	Observe the instructions for use	🏭	Manufacturer
🌡	Observe upper temperature limit	RUO	Research Use Only
Σ	Content sufficient for <n> provisions	BLOCK	PCR plate
BUFFER	FluoMix	CONTROL+	Positive control DNA
FOIL	PCR foil	CONTROL-	Negative control DNA
2	For single use only		

10. TROUBLE SHOOTING

Problem	Possible cause	Solution
General (can cause false positive and/or false negative/failure of reactions)	Incorrect amplification conditions	Check the thermal cycler. The cyclers are validated for carrying out the method: see points 6.3
	Evaporation due to leaking foil	Use the applicator to press down the film. Ensure a tight fit at the edges. Use a pressure mat during the PCR.
	Too long a period between PCR reaction and post-measurement	Perform the post-read max. 15 min. after the PCR program has finished.
	Liquid splashes on optical film	Change foil.
	Fogged or dirty optical film	Incubate the plate again in the thermal cycler with the lid heater switched on (approx. 30 seconds) or replace the foil. Only touch the foil with clean disposable gloves. Ensure that the FluoVista is not placed in the direct draught of the air conditioning system.

Problem	Possible cause	Solution
	Time between PCR preparation and pre-measurement longer than 15 minutes	Measure the plate within 15 minutes after PCR preparation.
Failure of reactions / false negative reactions	Too high volume used when loading the PCR plate	Check the pipette setting.
	Poor DNA quality or too low DNA concentration	Photometric determination of the degree of purity of the DNA. The quotient of the absorbances at λ=260 nm and λ=280 nm should be 1.8. Set the DNA concentration to 1 ng/µl. Dilute the DNA with fluorescence-free distilled water.
	Magnetic beads in the PCR assay	Separate beads via magnet or centrifugation.
	PCR inhibitors in distilled water.	Use fresh distilled water.
	Mastermix DNA mixture not sufficiently mixed	Vortex the Mastermix-DNA mixture thoroughly.
	Mastermix not pipetted into well	Visual inspection. After adding the master mix, the solution turns a bluish color.
	Mastermix pipetted into well with air bubbles	Optical control. If there are air bubbles, centrifuge the PCR plate.
	Plate does not sit correctly on FluoVista Carrier Plate during post-measurement	Check the plate for correct positioning before each read.
	Storage between PCR preparation and pre-measurement, or between thermal cycler and post-measurement in the light.	Measure the plate within 15 minutes after PCR preparation and always store in the dark.
False positive reactions	Insufficient volume used when loading the PCR plate	Check the pipette setting.
	DNA concentration too high	Determine the DNA concentration and adjust to 1 ng/µl. Dilute the DNA with fluorescence-free distilled water.
	Red or yellow pens used on plate	Only use black or blue pens for marking on the edge of the plate.
	Air bubble in the well during pre-measurement	Destroy potential air bubbles by sufficient centrifugation.

11. LITERATURE

1. Mullis KB, Faloona F: Specific synthesis of DNA in vitro via polymerase catalyzed 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.

3. Olerup O, Zetterquist H: HLA DR typing by PCR amplification with sequence specific primers (PCR SSP) in two hours: An alternative to serological DR typing in clinical practice including donor recipient matching in cadaveric transplantation. Tissue Antigens 1992;39:225-235.

4. Bunce M, O'Neill CM, Barnardo MCNM, Krausa P, Browning MJ, Morris PJ, Welsh KI: Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). Tissue Antigens 1995;46:355-367.

5. Bunce M, Young NT, Welsh KI: Molecular HLA Typing - The brave new world. Transplantation 1997; 64:1505-1513.

6. Holland PM, Abramson RD, Watson R, Gelfand DH. Detection of specific polymerase chain reaction product by utilizing the 5'-3' exonuclease activity of Thermus aquaticus DNA polymerase. Proc Natl Acad Sci USA 1991; 88:7276-7280.

7. isvikis S, Alexandra Schlenck A, Maurice M. DNA Extraction and Stability for Epidemiological Studies. Clin Chem Lab Med 1998; 36(8):551–555

8. Wen Shao, Sonny Khin, and William C. Kopp. Characterization of Effect of Repeated Freeze and Thaw Cycles on Stability of Genomic DNA Using Pulsed Field Gel Electrophoresis. 2012, Biopreservation and Biobanking, Vol. 10, No. 1