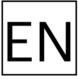
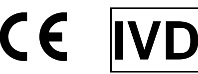


INSTRUCTIONS FOR USE

FastQ® B*27 direct

Test kit for determination of HLA-B*27 on a molecular genetic basis

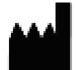
Electronic instructions for use see www.bag-diagnostics.com



REF 728201 FastQ® B*27 direct

Version: 5/2022 / Issued: 2022-06 Changes to version 3/2021 and 4/2022 are marked in orange.

If a complete chapter is new or changed only the headline is marked in orange



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1. INTENDED USE

The intended purpose of the FastQ® B*27 direct kit is the determination of the presence of HLA-B*27 alleles that are associated with different types of axial spondyloarthritis.

2. PRODUCT DESCRIPTION

The **FastQ® B*27 direct** kit is used for the molecular genetic detection of HLA-B*27 alleles. The HLA-B27 protein is a variant of the human leucocyte antigen-B (HLA-B). The HLA-B27 protein is associated with different autoimmune diseases (Bechterew's disease or Spondylitis ankylosans respectively, Reiter's disease, reactive arthritis) and is, therefore, used as part of the diagnostic procedure (1, 2). A positive HLA-B*27 result is associated with a very high disease risk. In suspected cases of M. Bechterew, a HLA-B*27 diagnosis provides an important contribution to the therapy of the patient. Around 3% to 6% of the people carrying the HLA-B*27 gene develop Spondylitis ankylosans and more than 90% of all patients with a seronegative arthritis are carrying this gene. The **FastQ® B*27 direct** kit covers all common HLA-B*27 subtypes. The test can be performed without DNA isolation directly from blood or buffy coat.

3. TEST PRINCIPLE

The test is performed with EDTA whole blood or buffy coat as starting material. The DNA that is released from the lymphocytes is amplified in a PCR with sequence-specific primers (SSP). The primers were specially developed for the selective amplification of the Exon 2 of the HLA-B*27 gene, which do only recognize the B*27 subtypes. The amplicons are detected with likewise gene locus specific fluorescent dye-labelled hydrolysis probes (TaqMan® probes), which increases the diagnostic sensitivity and specificity of the test compared to a conventional SSP. If amplicons are present, the probes are hydrolyzed by the Taq polymerase and a fluorescence signal is generated that increases proportionally to the amount of the PCR product. The fluorescence signals are measured by the optical detection unit of the RT-PCR-Cycler.

The test is performed in a single PCR reaction that detects the internal positive control (human HBB gene) and the disease-associated subtypes with different fluorescent colours.

4. MATERIAL

4.1 Contents of the FastQ® B*27 direct kit

- **1 x 260 µl Q Primermix B27-d**, ready to use, contains primers and probes
- **1 x 600 µl Q Mastermix**, ready to use, contains dNTPs,Taq Polymerase, reaction buffer
- **2 x 130 µl Blood Booster,** ready to use
- **Electronic instructions for use / kit file**, available from the download server www.service.bag-diagnostics.com, for further information see accompanying information sheet in the kit.

4.2 Additionally required reagents and devices

- Validated Real-Time PCR-Cycler and matching reaction tubes
- Aqua dest.
- Piston pipettes (0,5 – 1000 µl) and tips
- Colour Compensation kit for LightCycler® 480 II (REF 728259 RT CC Universal LC480, provided by BAG Diagnostics)

4.3 Validated cyclers and reaction tubes

Cycler	RT-PCR reaction tubes	RT-PCR closing systems
CFX96 Touch™ & CFX Opus 96 Real-Time PCR Detection System, Comp. Bio-Rad	FrameStar® Breakable Vertically PCR Plate, Low Profile Product No. 4ti-1201 Removable 8 Well PCR Tube Strip, Product No. 4ti-0753 Comp. Azenta Life Sciences	Strip of 8 Flat Optical Caps Crystal Clear, Product No. 4ti-0755 qPCR Adhesive Seal Product No. 4ti-0560 Comp. Azenta Life Sciences
LightCycler® 480 II Real-Time PCR Detection System, Comp. Roche Molecular Systems Inc.	Light Cycler® Multiwell Plate 96, white Product No. 04729692001 Comp. Roche Molecular Systems Inc.	qPCR Adhesive Seal Product No. 4ti-0560 Comp. Azenta Life Sciences
QuantStudio™ 6 Flex Real-Time PCR System, Comp. Applied Biosystems / Thermo Fisher Scientific	Removable 8 Well PCR Tube Strip, Product No. 4ti-0753 Comp. Azenta Life Sciences Saphire PCR Microplate, 96 Well, semi skirted, ABI design Product No. 652260 Comp. Greiner BIO-ONE®	Strip of 8 Flat Optical Caps Crystal Clear, Product No. 4ti-0755 qPCR Adhesive Seal Product No. 4ti-0560 Comp. Azenta Life Sciences

Note: If other realtime cyclers, reactions tubes and closing systems are used they must be validated by the user.

5. STORAGE AND STABILITY

The FastQ® B*27 direct kits are shipped with dry ice. Upon receipt store all reagents in temperature monitored devices at ≤ -20 °C. The expiry date is indicated on the label of each reagent. The expiry date indicated on the outer label refers to the reagent with the shortest stability contained in the kit. The freeze-thaw cycle testing has shown that up to 15 cycles for the Q Primermix B27-d and the Q Mastermix and up to 12 cycles for the Blood Booster have no detrimental effects on the quality of the kit.

If the protective packaging is damaged, please contact the customer service.

The pipetted reaction mixture before or after addition of the diluted blood sample can be stored protected from light at 2...8°C for up to 20 hours before starting the PCR run.

6. TEST PROCEDURE

6.1 Safety conditions and special remarks

Molecular genetic techniques are particularly sensitive and should be performed by well trained personnel experienced in molecular genetic techniques. The results of these tests must not be used as sole basis for diagnostic and/or clinical decisions.

Special safety conditions must be observed in order to avoid contamination and thus false reactions:

- Wear gloves during work (powder-free, if possible).
- Use new tips with each pipetting step (with integrated filter).
- If possible, use separate working areas for pre-amplification (DNA isolation and PCR set up) and post-amplification (detection).
- Use devices and other materials only at the respective places and do not exchange them.

6.2 Sample preparation

EDTA whole blood or buffy coat has to be used as sample material. Blood samples should be stored at maximum for 9 days at ambient temperature followed by 3 days at 2...8°C.

The samples have to be **mixed thoroughly** and diluted as follows:

➔ Dilution 1:50: **5 µl** whole blood / buffy coat + **245** µl A.dest.

6.3 Amplification

Reaction tubes recommended by the manufacturer of the realtime cycler or the materials recommended in chapter 4.3 should be used.

The reaction volume for each RT-PCR test is 10 µl.

For each sample the following reagents are pipetted into a reaction tube:

2 µl	Q Primermix B27-d
5 µl	Q Mastermix
1 µl	Blood Booster
1-2 µl	Sample material (diluted 1:50 in Aqua dest.)
0-1 µl	Aqua dest. (depending on volume of sample material)

The samples have to be mixed thoroughly before setting up the test!

For the **negative control (NTC)**, prepare a PCR reaction with Aqua dest. instead of the sample material.

If a premix of Q Primermix B27-d, Q Mastermix, Blood Booster and Aqua dest. is prepared for more than one sample please allow for a reasonable additional amount for pipetting losses.

Close the reaction tubes and briefly spin down the liquid. Ensure that no bubbles are present in the wells. If bubbles are observed, gently tap assay on the bench to remove the bubbles.

6.4 Setup of the RT-PCR cycler

The following fluorophores are used for the **FastQ® B*27 direct** kit:

Fluorophor	Wave lenght in nm	
FAM	Excitation: 495	Emission: 520
CAL Fluor® Orange 560	Excitation: 538	Emission: 559

6.4.1 CFX96 Touch™ & CFX Opus 96 Real-Time PCR Detection System

Note: The colour names in the CFX software must not be changed. The PlexTyper® software need the default names for the interpretation and the correct import.

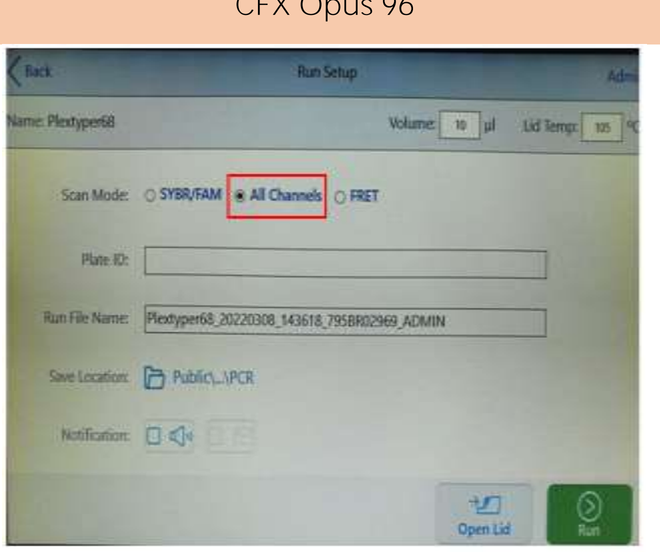
Channel	Fluorophore	Selected
1	FAM	☑
	SYBR	☐
2	HEX	☑
	TET	☐
	Cal Orange 560	☐
	Cal Gold 540	☐
	VIC	☐
3	ROX	☐
	Texas Red	☑
	Cal Red 610	☐
	Tex 615	☐
4	Cy5	☑
	Quasar 670	☐
5	Quasar 705	☑
	Cy5-5	☐

PCR program:

Step	Time [s]	Temperature [°C]	Ramp rate [°C/s]	Plate read	Cycles
Initial activation	120	96	2,5	-	1
Denaturation	5	98	2,5	-	18
Annealing + Extension	25	64	2,2	-	
Denaturation	5	98	2,5	-	42
Annealing + Extension	25	64	*-	yes	

* use the default ramp rate of the CFX96 Touch™ & CFX Opus 96 Real-Time PCR Detection System

Note: Before starting the program choose the correct Scan Mode: All Channels. If the wrong Scan Mode is used the test cannot be interpreted and must be repeated. The lid temperature must be set to 105°C.

CFX96 Touch™	CFX Opus 96
	

6.4.2 LightCycler® 480 II Real-Time PCR Detection System

Please note that the light source for this cycler has been changed. From serial number 29001 it is an LED lamp, previously it was a xenon lamp. The test was validated on a unit with an LED lamp. It is expected that the older versions will also be compatible with the test, but it is likely that colour compensation will be required. Please contact BAG Diagnostics if you have a device with a xenon lamp and your results are suboptimal.

PCR-Program

According to the operating instructions of the LightCycler® 480 II, create and save a PCR protocol with the following parameters:

Detection Format: FastQ® B*27 direct, Block size 96, Reaction volume 10 µl

Step	Cycles	Analysis Mode	Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp rate (°C/s)
Hold	1	None	96	None	00:02:00	2.5
Cycle	18	None	98	None	00:00:05	2.5
			64	None	00:00:25	2.2
Cycle	42	Quantification	98	None	00:00:05	2.5
			64	Single	00:00:25	2.2

Channels for the LightCycler® 480 II Real-Time PCR Detection System

Please use the following channel settings in **Detection Format**:

Excitation	Emission 488	510	580	610	640	660
440						
465		✓				
498						
533			✓			
618						

Excitation Filter	Emission Filter	Name	Melt Factor	Quant Factor	Max Integration Time (Sec)
465	510	FAM	1	10	1
533	580	O560 (CalFluor Orange560)	1	10	1

It is strongly recommended to perform the device-specific colour compensation with the RT-CC Universal LC480 Kit (REF 728259) and to use it in PlexTyper® to correct the coefficients. If you have any questions regarding this, please contact BAG Diagnostics customer service at info@bag-diagnostics.com or +49 6404 925125.

Please note the device settings for the plate type: White Plates / Clear Plates

6.4.3 QuantStudio™ 6 Flex System

Experiment properties:

Instrument type:	QuantStudio™ 6 Flex System
Block type:	96-Well (0.2 mL) oder Fast 96-Well (0.1 mL)
Experiment type:	Comparative Ct (ΔΔCt)
Reagent type:	TaqMan® Reagents
Run properties:	Standard

Define Targets:

Target Name	Reporter	Quencher	Color
FAM	FAM	NFQ-MGB	Green
ORANGE560	VIC	NFQ-MGB	Orange

Passive Reference: None
Assignment: Assign all targets to each well.
Reaction volume: 10 µl

Run Method:

Stage	Cycles	Data Collection	Target (°C)	Hold (mm:ss)	Ramp rate (°C/s)
Hold Stage	1	Off	96	00:02:00	2.5
PCR Stage	18	Off	98	00:00:05	2.5
			64	00:00:25	2.2
PCR Stage	42	Off	98	00:00:05	2.5
		On	64	00:00:25	2.2

7 EVALUATION AND INTERPRETATION OF RESULTS

7.1 Interpretation with the PlexTyper® Software

The evaluation and interpretation of the test results can be carried out with the PlexTyper® software when using the validated RT cyclers listed below. Please also refer to the instructions for use for the PlexTyper® software.

- CFX96 Touch™ and CFX96 Opus Real-Time PCR Detection System, Bio-Rad
- LightCycler® 480 II Real-Time PCR Detection System, Roche Molecular Systems Inc.
- QuantStudio™ 6 Flex System, Applied Biosystems / Thermo Fisher Scientific

Creating tests and worklists in PlexTyper® is described in detail in the Instructions for Use for the PlexTyper® software.

When using other RT cycler systems, manual evaluation and interpretation must be performed as described in section 7.2.

For software-based evaluation and interpretation of the data, the PlexTyper® software (available free of charge from BAG Diagnostics) is required in conjunction with the PlexTyper® specific kit files. The kit files required for the evaluation are available for download on the download server (www.service.bag-diagnostics.com).

Note the product and lot number of the kit used. The kit files are product and lot specific and also specific for the RT-PCR cycler used. The use of wrong kit files (wrong kit, wrong lot, wrong cycler) can lead to incorrect genotyping.

To evaluate the results, the data must be transferred from the thermal cycler to a computer with the PlexTyper® software (e.g. with a suitable USB stick). Please refer to the PlexTyper® instructions for use for data evaluation.

It is possible, but not necessary, to check the data generally in the thermocycler software. For example, valid tests must show sufficient fluorescence signals in the FAM channel of the internal control. Positive reactions show a positive colour signal in the corresponding colour channel.

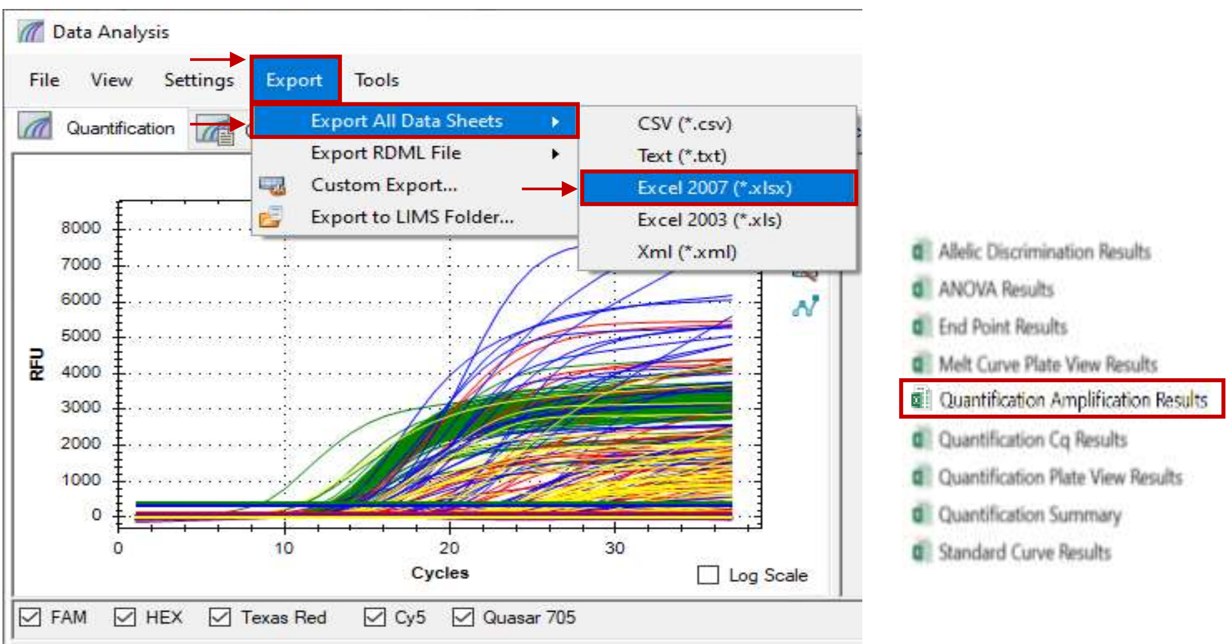
A negative control (NTC) serves as a contamination control. If DNA or contaminating amplicons are unintentionally added to the NTC reaction, this leads to a positive signal. If the Cq is below 36, this indicates possible contamination. Amplification signals with a higher Cq than 36 in the NTC can be regarded as PCR artefacts. If PCR contamination is suspected, it is recommended to decontaminate the PCR laboratory from DNA and to exchange the reagents.

The raw data determined by the cycler-specific software are imported into the PlexTyper® software. For this, an export of the cycler-specific raw data must be carried out in advance, as described in section 7.1.1. The PlexTyper® software uses the Cq values, RFUs (Relative Fluorescence Units) and the shape of the amplification curve to determine the positive and negative reactions from which the molecular genetic HLA B*27 characteristics of the samples used are determined.

7.1.1 Export of results from the cycler

7.1.1.1 CFX96 Touch™ & CFX Opus 96 Real-Time PCR Detection System

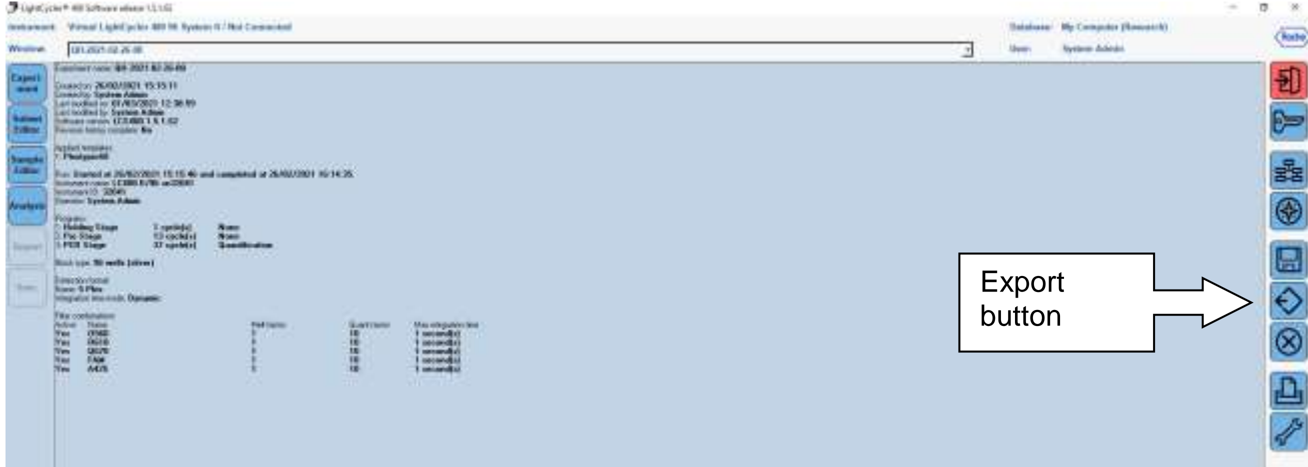
Open the data file with the CFX software and export the Excel 2007 file (.xlsx).



Note: Only the file "Quantification Amplification Results" is needed. It makes sense to delete the other files.

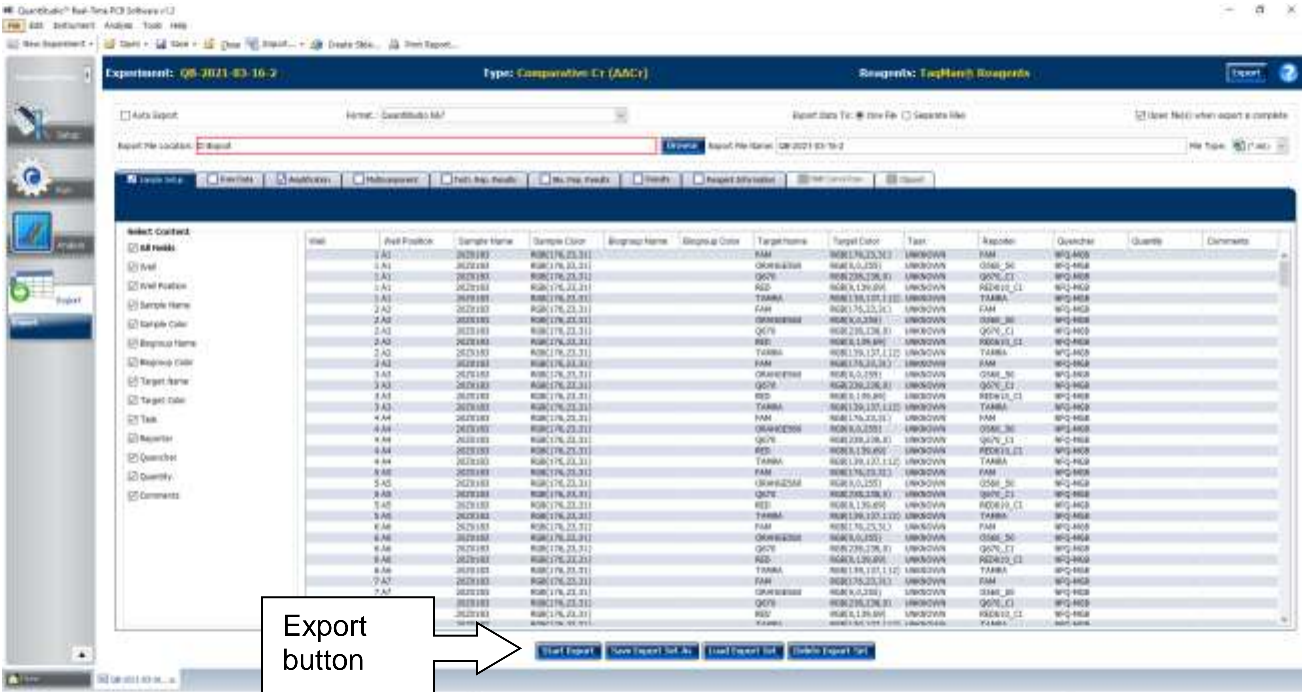
7.1.1.2 LightCycler® 480 II Real-Time PCR Detection System

PlexTyper® uses xml files from the LightCycler® 480 II. After the run no analysis in the Roche software is needed. Export the raw data in XML format.



7.1.1.3 QuantStudio™ 6 Flex System

Open the Export menu and start the export of the "Sample Setup" and the "Amplification" tab as (*.xls) file.



7.1.2 Evaluation and interpretation

The PlexTyper® software receives raw data from the amplification files of the supported real-time devices and calculates the data for the Cq value. It also analyses the quality of the amplification and automatically assigns positive and negative reactions based on this.

The PlexTyper® kit files contain the threshold values for the reactions and the HLA-B*27 specificities for each reaction in each colour channel. The possible HLA-B*27 genotypes are calculated from the pattern of positive and negative reactions. The possible genotypes are displayed to the user and the user can accept or edit the genotype.

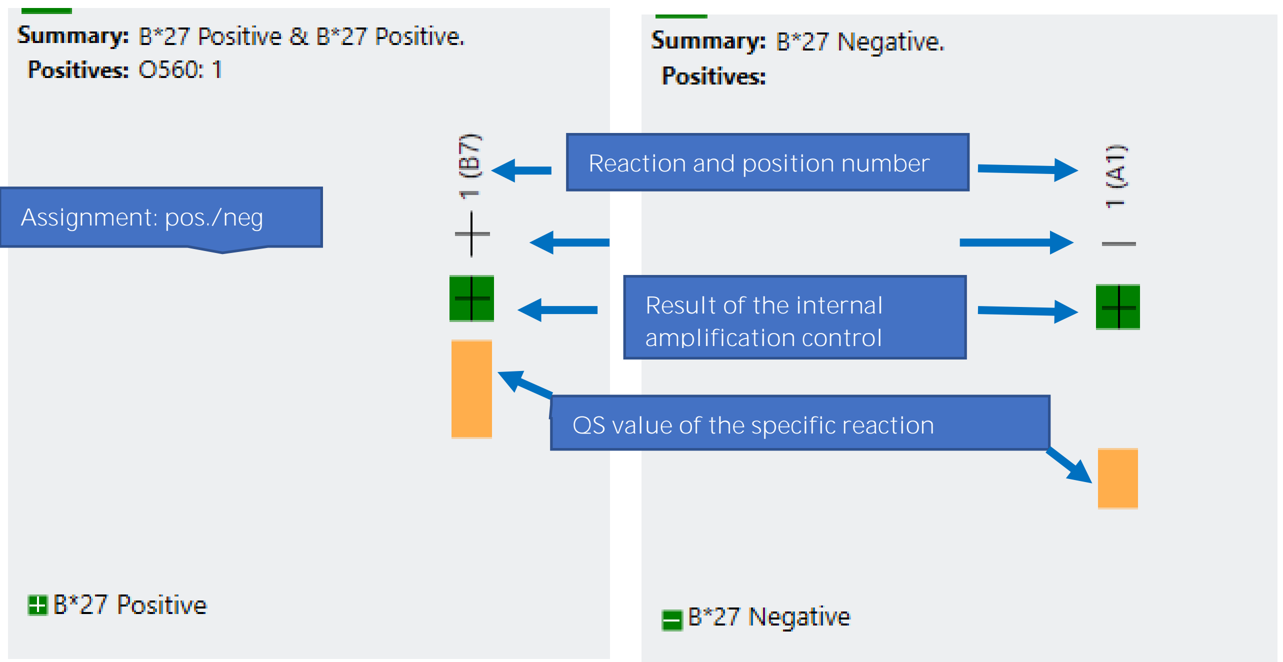
The HLA-B*27 genotypes can be filtered so that only the common alleles (Common) or the common and well documented alleles (Common and Well Documented = CWD) are displayed. Alternatively, all alleles of the IMGT database used in the kit file can be displayed. The recommended default setting is CWD format.

Common alleles are shown in green, well-documented alleles in blue and rare alleles (only with the All option) in grey. The CWD list is based on the CWD 2.0.0 catalogue (Mack et al. 2013), but some entries have been changed due to more recent sequence data (see CWD 2.1.0 list in the download section of the BAG Diagnostics website: [Downloads on our BAG In-vitro technologies and products (bag-diagnostics.com).](bag-diagnostics.com) The Common filter reduces the displayed alleles to the common alleles. If All is selected, all alleles including the rare ones (shown in grey) are displayed.

7.1.2.1 Result histogram

The result histogram shows all reactions for a test. The colour of the bars indicates the colour channel in which the reaction is detected. The green field above the histogram represents the internal amplification control. If this fails, the field turns white and contains a "-". The + - buttons in the upper right corner can be used to enlarge or reduce the histogram.

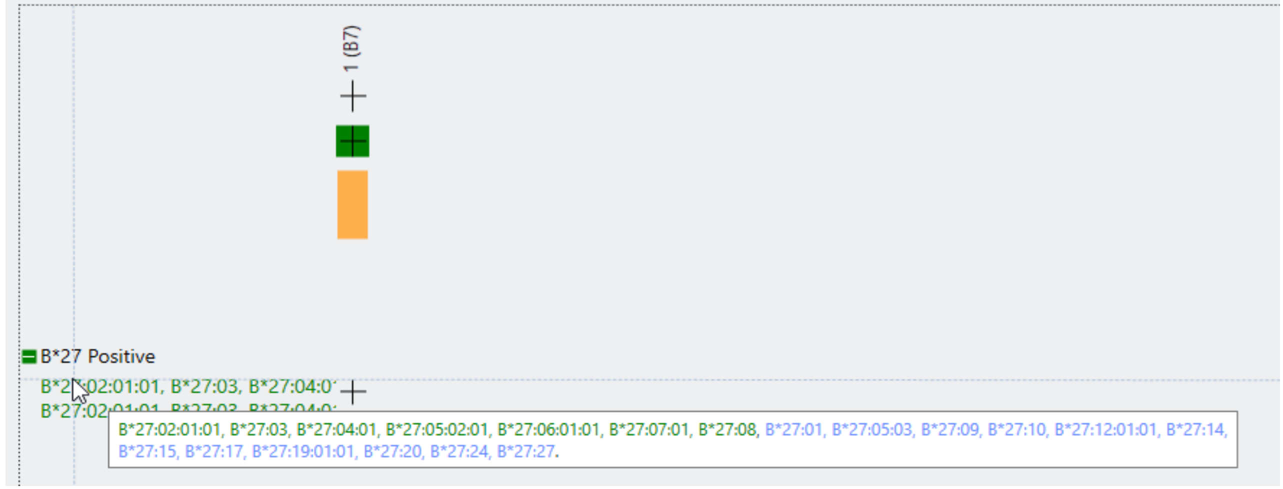
Basically, there are only two options for the FastQ® B*27 direct kit: B*27 positive or B*27 negative:

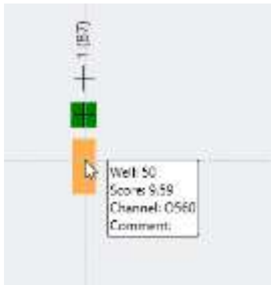


When the mouse is moved over the histogram, a window opens with additional information (e.g. the QS value). The height of the bar corresponds to the quality score (QS value) of the reaction. Positive reactions are directed upwards, negative ones downwards. The higher the bar, the more clearly positive or negative a reaction is rated. A detailed description of the QS values can be found in the user manual for the PlexTyper® software.

Above the histogram is a summary of the result: B*27 Positive & B*27 Positive or B*27 Negative and a mention of the positive reaction ("Positives"). The representation as B*27 Positive & B*27 Positive comes from the evaluation of complete HLA typings in the same software and is not entirely correct, as it can be a homozygous or a heterozygous result for B*27. With the Concatenate alleles function (not activated) alleles can be combined. This function can be helpful if the All option including rare alleles has been selected.

By default, the results are summarised in the histogram as B*27 positive or B*27 negative. In case of positive results, the button expands the results and shows the allele combinations with the respective reaction patterns. By moving the mouse over the allele combination, a complete list of alleles is displayed, which can be very long if the All Filter is selected.

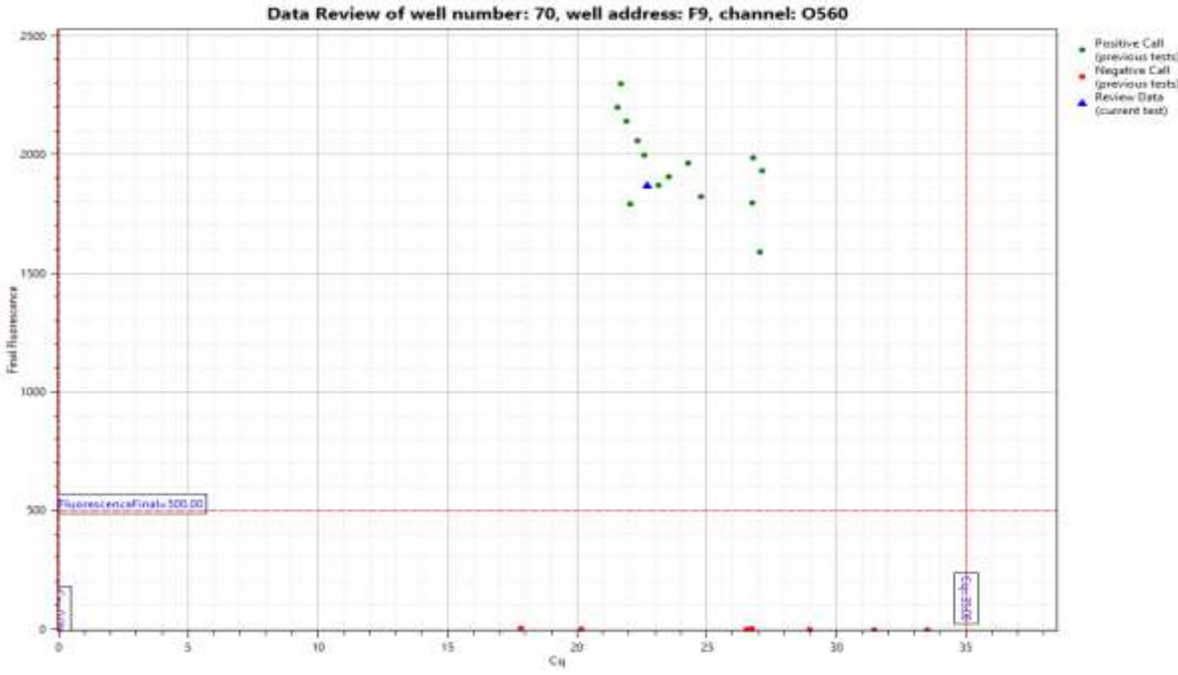




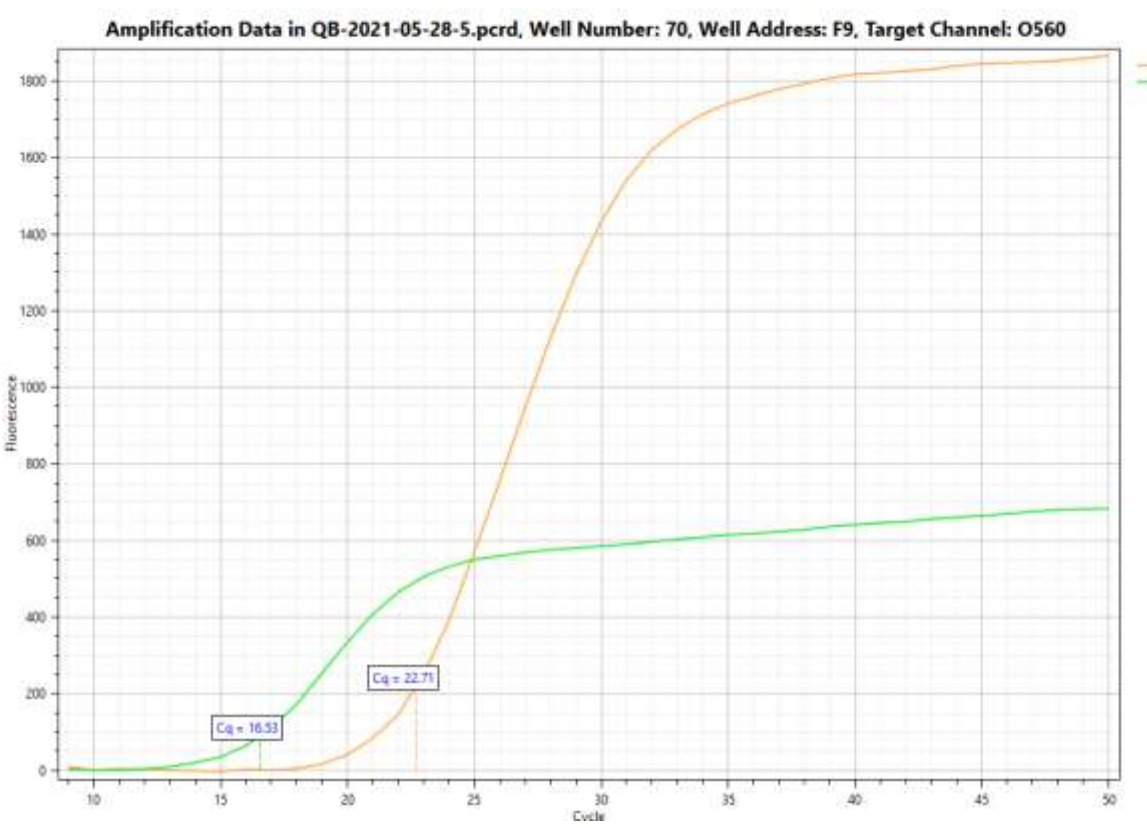
7.1.2.2 Interpretation tools

There are some tools available in PlexTyper® that can be useful when the automatic interpretation does not find a result or if there is a rare result that should be checked. Most of these tools are intended for complete HLA typing and are not useful for the evaluation of the FastQ® B*27 direct kit. A detailed description can be found in the Instructions for Use for the PlexTyper® software. In general, reactions with a poor quality score (between +3 and -3) should be checked.

Double-clicking on bars for the QS value opens a diagram showing the Cq value and the final fluorescence of the reaction in the context of other reactions with the same kit lot:

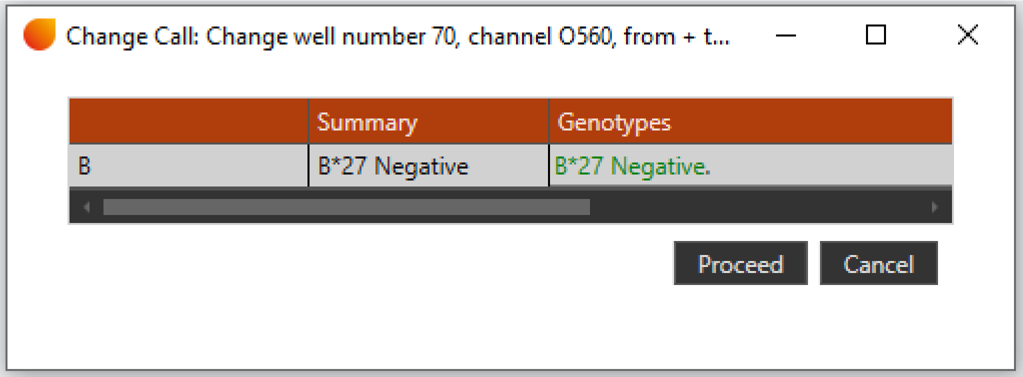


The red lines indicate the thresholds for positive reactions. The blue triangle represents the currently selected test. Double-clicking on the blue triangle opens a window with the amplification curves for the internal amplification control (green) and the B*27 specific reaction (orange). In case of a poor QS value, check whether the reaction is close to one of the threshold values and whether the amplification curve has a sigmoidal shape.

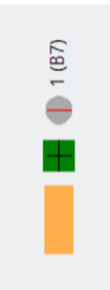


7.1.2.3 Change a reaction call

If the software has incorrectly assessed a reaction with a poor QS value, this assessment can be changed manually. All changes by the user are logged and displayed in the audit trail in the results report. With a right mouse click on the corresponding bar in the histogram, a preview of the effect of a change in the reaction can be opened (Preview effect of change from + to - or in the other direction). Then select either Proceed to change the result or Cancel to discard the change.

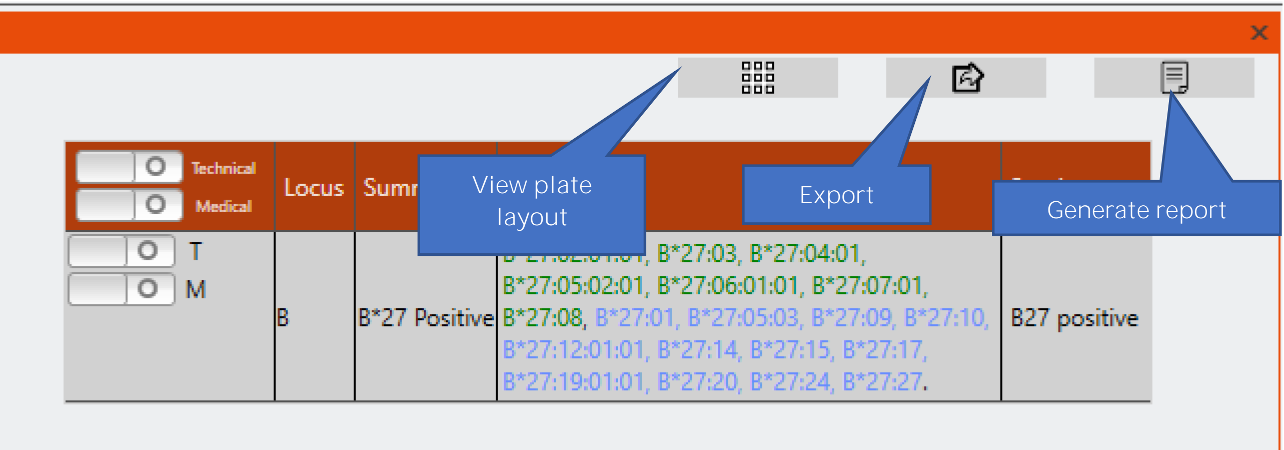


A changed reaction is shown in red in the histogram as shown in the figure.



7.1.3 Results view

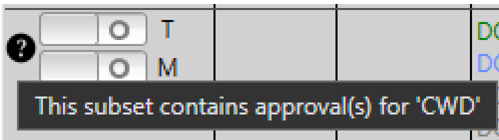
On the right side of the screen, the results are displayed in a table containing the following information: Results Summary, Genotype as a complete list of possible alleles (reflecting the selected filter), Accepted Phenotype (serological equivalent) and the approval status. From this table, the results can be exported to a text file using the Export button and a PDF report can be generated using the Create Report button. The View Plate button displays the plate layout as an image that can be copied to the clipboard..



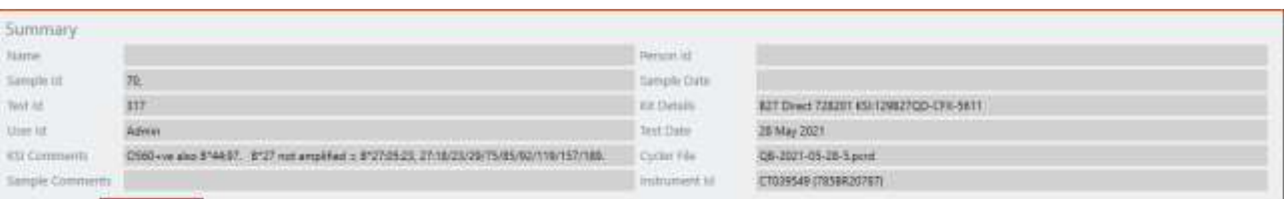
In the Reaction Comments field, comments generated by the software regarding the efficiency of the test are displayed. In another field at the bottom of the window, comments can be entered by the user (User annotation of result).

A two-step approval process is implemented in the software. The Technical approval -T can be done either by a user with the role Technician or Supervisor. To do this, the button in the first column is pressed and it then turns green. In a second step, the Medical approval (-M) is carried out exclusively by a Supervisor. The approval can be done for all gene loci together in the header line or individually in the respective lines with the gene loci.

If a gene locus has been approved (either technically or medically) and a reaction score is subsequently changed for this result, the approvals are all removed (initially a warning is displayed). If the CWD filter is changed, the clearances with the originally selected filter will be retained (a "?" icon will appear next to the gene locus indicating that there is a clearance with a different filter). If the user tries to approve this gene locus with another filter, the original approvals will be removed. Approvals with different filters for the same result are not possible, i.e. a result can only be approved with one filter or not at all..



In the header above the result histogram, information on the sample, the kit used and the cycler used is given. Under KSI Comments there is the information that in case of a positive reaction the rare allele B*44:97 cannot be excluded and that some rare B*27 alleles are not covered by the kit. This information also appears in the results report.



7.2 Manual interpretation of results

All tests with released human gDNA must show a fluorescence signal in the green channel (FAM) with the internal control. HLA B*27 positive samples show a positive signal in the channel for CAL Fluor® Orange 560. Amplification signals for HLA B*27 negative samples

must be outside the defined Cq values for the CAL Fluor® Orange 560 channel. A negative control (NTC) with Aqua dest. should not show any fluorescent signal during the complete RT-PCR run and represents a contamination control.

Fluorescence signals within the defined Cq values with the negative control with Aqua dest. indicate contamination. Flourescence signals outside the defined Cq values can occur due to the very sensitive test method in case of inaccurate pipetting. If this occurs, the test should be repeated. Furthermore, a detailed analysis is recommended. If necessary, the PCR working place has to be decontaminated and the reagents have to be exchanged.

The following signals are rated as positive:

Specificity	Fluorophor	Cq level	Wavelength (nm)
B*27	CAL Fluor® Orange 560	< 30	Excitation: 538 / Emission: 559
Internal control	FAM	< 20	Excitation: 495 / Emission: 520

The Cq level defines the latest Cq number when a positive reaction (fluorescence rises above the threshold) is expected in the respective channel. The threshold that is automatically set by the cycler software should be used as baseline threshold. It is recommended to check the plausibility of the reactions with the amplification curves and to repeat questionable results. If there are questions regarding the adaptation of the threshold or borderline Cq values please contact the technical support of BAG Diagnostics (phone: +49 (0)6404 925125, email: info@bag-diagnostics.com).

7.3 Specificity of the kit

The follwing alleles are recognized by the kit:

Fluorophor	Common*	Well documented*	Rare*
CAL Fluor® Orange 560 (B*27 positiv)	B*27:02:01:01, *27:03, *27:04:01, *27:05:02:01, *27:06:01:01, *27:07:01, *27:08,	B*27:01, *27:05:03, *27:09, *27:10, *27:12:01:01, *27:14, *27:15, *27:17, *27:19:01:01, *27:20, *27:24, *27:27	B*27:02:01:02-*27:02:06, *27:04:02-*27:04:06, *27:05:02:02- *27:05:02:32, *27:05:04-*27:05:56, *27:06:01:02, *27:07:02-*27:07:06, *27:11, *27:12:01:02-*27:12:01:03, *27:13:01-*27:13:02, *27:16, *27:19:01:02, *27:21:01-*27:21:02, *27:25, *27:26, *27:28 , *27:30-*27:74, *27:76-*27:84, *27:86-*27:91, *27:93 -*27:118, *27:120-*27:156, *27:158-*27:188, *27:190-*27:203-*27:255 / B*44:97

Rare B*27 alleles not detected: B*27:05:23, B*27:18, B*27:23, B*27:29, B*27:75, B*27:85, B*27:92, B*27:119, B*27:157, B*27:189

IMGT Database 3.47.0

* Common and well documented Allele by CWD 2.1.0 catalogue

8 WARNINGS AND PRECAUTIONS

The **FastQ® B*27 direct** is designed for in vitro diagnostic use and should be used by properly trained, qualified staff only. All work should be performed using Good Laboratory Practices.

Biological material used for the test, e.g. blood, should be handled as potentially infectious. When handling biological material appropriate safety precautions are recommended (do not pipet by mouth; wear disposable gloves while handling biological material and performing the test; disinfect hands when finished the test).

Biological material should be inactivated before disposal (e.g. in an autoclave). Disposables should be autoclaved or incinerated after use.

Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a suitable standard disinfectant or 70% alcohol.

Material used to clean spills, including gloves, should be inactivated before disposal (e.g. in an autoclave).

Disposal of all samples, unused reagents and waste should be in accordance with country, federal, state and local regulations.

Microbial contamination of the reagents while taking aliquots should be avoided. It is recommended to use sterile one way pipettes and tips. Reagents that look cloudy or show any signs of microbial contamination must not be used.

A Material Safety Data Sheet resp. a declaration on Material Safety Data Sheets (MSDS) is available to download at www.bag-diagnostics.com.

9 SPECIFIC PERFORMANCE CHARACTERISTICS

The combination of primers and probes ensures a reliable identification of the B*27 alleles specified in chapter 7.3. The accuracy and reproducibility of the specificity of the test kit is verified for each lot with pre-typed reference samples.

To define the diagnostic sensitivity and specificity of the **FastQ® B*27 direct** kit performance evaluation studies with pre-typed blood samples were performed. The results were compared to the results that were obtained with other CE certifed typing reagents (amongst others serology, SSO, SSP) and/or sequencing. No discrepancies in the detection of the B*27 feature have been observed (100% concordance).

Blood samples	Internal and external study total (CFX)	Internal study total (QS6)	Internal study total (LC480II)	Percentage concordance [%]
B*27 negative	79	84	84	100
B*27 positive	17	17	17	100
Total	96	101	101	100

Summary of the internal and external study results for the Q Primermix B27-d with percentage concordance to the reference typing and detection of HLA-B*27

Additionally, the stabilizing effect of the Blood Booster especially on fresh blood samples has been shown with six pre-typed blood samples. No discrepancies were observed for the HLA-B*27 feature and the variance in the Cq values could be reduced significantly.

10 LIMITATIONS OF THE METHOD

Because of the high susceptibility of the RT-PCR method for cross contaminations special care should be taken during sample preparation. Validation tests in the course of the performance evaluation study of the **FastQ® B*27 direct** kit have shown that a sample dilution between 1:400 and 1:25 do not have a significant influence on the detection of the B*27 alleles. It should be made sure that the sample material is thoroghly mixed to ensure that enough cells with a nucleus are available for the PCR reaction. If this is not done there might be false negative results in the B*27 specific colour channel.

Extreme care should be taken to prevent contamination of the kit reagents and other laboratory materials and equipment with amplicons, DNA or blood samples. The negative control with Aqua dest. (NTC), which is carried out with every test run, serves as a contamination control.

In the negative control with Aqua dest. there must not be any fluorescent signal (Cq > N.A.). In the case of signal development with the negative control the PCR working place has to be decontaminated and the reagents have to be exchanged if necessary.

Regular wipe tests (e.g. BAG Wipe Test, REF 7091) are strongly recommended.

All instruments (e.g. pipettes, realtime cyclers) must be calibrated according to the manufacturers instructions.

11 INTERNAL QUALITY CONTROL

Internal quality control of new lots of the **FastQ® B*27 direct** kit can be performed using a combination of samples with known HLA type. An internal positive control for successful amplification is contained in the Q Primermix.

A negative control is carried out to detect possible contamination. For this purpose, a PCR reaction without sample material is set up (NTC).

12 TROUBLESHOOTING

Symptom	Possible reason	Potential solution
Bad or no signal	Presence of an inhibitor	Use fresh reagents
	No gDNA in the reaction	Repeat test Take care of correct pipetting and dilution of the blood samples
	Wrong amplification parameters	Check PCR program and ramp rate
	Fluorescent probes or primers degraded	Use fresh Q Primermix Avoid exposition to light and frequent thawing and freezing. Observe storage conditions!
	Bubbles in the PCR reaction, remaining liquid at the inner wall of the tube	Careful pipetting Spin down PCR plate
	Incompatible or low quality RT-PCR plastic ware	Use compatible and high quality plastic ware (see chapter 4.3)
	Wrong signal calculation due to abnormal amplification signals during the initial cycles of the run	Application of corrective measures in the software (e.g. "apply fluorescence drift correction" function from Bio-Rad or exclusion of the first five cycles from analysis)
	Evaporation of the reagents due to incorrect closing of the PCR tubes	Make sure that the PCR tubes are closed properly Be careful at the edges of sealing foils
Signal in the negative control	Contamination with DNA or blood in the negative control	Repeat the negative control Decontaminate the workplace

13 TRADEMARKS USED IN THIS DOCUMENT/PRODUCT

TaqMan® is a registered trade mark of Roche Molecular Systems Inc.

®Cal Fluor Dyes are registered trade marks used by the company LGC Biosearch Technologies

QuantStudio™ is a trade mark by the company Applied Biosystems / Thermo Fisher Scientific.

CFX96 Touch™ is a trade mark by the company Bio-Rad

LightCycler® is a registered trade mark by the company Roche Molecular Systems Inc.

FrameStar® is a registered trade mark by the company Azenta Life Sciences

14 EXPLANATION OF SYMBOLS USED ON THE LABELS

Symbol	Meaning
Σ	Sufficient for n tests
	Storage temperature / Upper limit of temperature
	Use by
	Consult instructions for use
	Manufacturer
BLOOD BOOST	Bood Booster, reagent for RT-PCR kits for detection directly from whole blood or buffy coat
CONT	Contens, contains
GENOTYPING	Intended use: Typing of human genetic markers that are associated with diseases or pharmacogenetic reactions
eIFU	Electronic instructions for use
IVD	For in vitro diagnostic use
LOT	Batch code
Q MASTERMIX	Mastermix for RT-PCR kits for detection directly from whole blood or buffy coat
Q PRIMERMIX | B27-d	Primermix for typing HLA-B*27 with the **FastQ® B*27 direct** kit
REF	Catalogue number

15 LITERATURE

1. Brewerton, DA et al., 1973. Lancet **i**:904-907
2. Schlosstien L et al., 1973. N. Engl. J. Med. 288:704-706
3. Mack et al., 2003, Tissue Antigens 81: 194-203

Instructions for use in other languages see http://www.bag-diagnostics.com
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