EN

Instructions for Use

FastQ B*27

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

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

IVD

REF 728208 FastQ B*27

CE

Contents

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1. INTENDED USE

The intended use of the FastQ product line is human genetic testing for markers that are associated with diseases or pharmacogenetic reactions. For the FastQ B*27 kit this is the determination of the presence of HLA-B*27 alleles that are associated with certain autoimmune diseases (see Product Description).

2. PRODUCT DESCRIPTION

The **FastQ B*27** 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 kit** covers all common HLA-B*27 subtypes. Moreover, the kit differentiates between the disease associated alleles and the subtypes HLA-B*27:06 or HLA-B*27:09, which are not associated with Spondylitis ankylosans (3).

3. TEST PRINCPLE

The test is performed with genomic DNA as starting material. The DNA is amplified in a PCR with sequence-specific primers (SSP). The primers were developed for the selective amplification of specific sequences in the exons 2 and 3 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), the disease-associated subtypes and the non-disease-associated subtypes with different fluorescent colours.

4. MATERIAL

4.1 Contents of the FastQ B*27 kit

- **260 µl Q Primermix B27**, ready to use, contains primers and probes
- **260 µl Q Plex Mix**, ready to use, contains dNTPs,Taq Polymerase, reaction buffer
- **Instructions for use**

4.2 Additionally required reagents and devices

- Reagents for DNA isolation (validated DNA isolation kits see 6.2)
- Real-Time PCR-Cycler (validated cycler see 4.3)
- RT-PCR reaction tubes with caps or foils (validated products see 4.3)
- Aqua dest.
- Piston pipettes (0,5 – 1000 µl) and tips
- Colour Compensation kit for LightCycler® 480 II (provided by BAG Diagnostics)

4.3 Validated cyclers and reaction tubes

Cycler	RT-PCR reaction tubes	RT-PCR closing system
CFX96 Touch™ Real-Time PCR Detection System Comp. Bio-Rad	FrameStar® Break-A-Way PCR Plate 96 white wells, black frame Product No. 4ti-1201 Fa. 4titude / Brooks Life Sciences	4titude Crystal Strips, Product No. 4ti-0755 Optically clear adhesive film, Product No. 4ti-0560 Comp. 4titude/Brooks Life Sciences

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

4.4 Recommendations for not validated cyclers and reactions tubes

For below mentioned cyclers initial tests have been performed but no full validation. The table contains the recommended specifications.

Cycler	RT-PCR reaction tubes	RT-PCR closing system
LightCycler® 480 System, Comp. Roche	LightCycler® 480 Multiwell Plate 96, white, Product No. 04729692001, Comp. Roche	LightCycler® 480 Sealing Foil, Product No. 04729757001 Comp. Roche
MIC (Magnetic Induction Cycler) Comp. Bio Molecular Systems	Tubes and caps: MIC-TUBES No. 68MIC-60653 Exclusive distributor (D/A): Comp. Biozyme	
Rotor-Gene Q Comp. Qiagen	Strip Tubes and Caps, 0.1 ml, Cat No./ID: 981103 or 981106, Comp. Qiagen	
Lightcycler 2.0 Comp. Roche	n.a.	

5. STORAGE AND STABILITY

The kits are shipped with blue 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 12 cycles for the Plex Mix and up to 15 cycles for the Q Primermix B27 have no detrimental effects on the quality of the kit. No data are available yet for more cycles. Therefore, it is recommended to aliquot the reagents if necessary.

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 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 DNA Isolation

The sample material for the isolation of genomic DNA must be sent in appropriate blood collection systems. For the test EDTA or Citrate blood is required. The presence of heparin potentially inhibits PCR; therefore blood collection systems with heparin are not suitable (4) and must not be used.
It is recommended to use CE IvD certified kits for the DNA isolation.

Validated DNA isolation kits:

- Qiagen QIAamp DNA Blood Kits (columns)
- Chemagic™ 360 (chemagic DNA Blood Kit, beads)

If the established standard method of the lab is used for gDNA isolation and this is not one of the validated kits above, it must be validated by the user.

A DNA concentration of 10 – 20 ng/µl is required to perform the FastQ B*27 test.

The DNA must have the following purity indexes:

- OD_{260}/OD_{280} = > 1.5 and < 2.0
  Higher values are an indicator for contamination with RNA, lower values for a contamination with proteins.
- OD_{260}/OD_{230} = > 1.8
  Lower values indicate a contamination with salt, carbohydrate or organic solvents.

6.3 Amplification

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

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

2 µl Q Primermix
2 µl Plex Mix
1 µl Sample DNA (10 – 20 ng/µl)
5 µl Aqua dest.

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

Recommended exceptions
Rotor-Gene Q Cycler **If the Rotor-Gene Q cycler is used a higher reaction volume must be used. The reaction volume must be set to 20 µl in the cycler settings.** **There are three possibilities for the PCR setup:** a) Use the double amount of the volumes given above resulting in 20 µl reaction volume. b) Add additional 5 µl Aqua dest. (15 µl in total). c) Overlay the PCR mix with 5 µl mineral oil (15 µl in total).
Lightcycler 2.0 **If the Roche LC 2.0 Cycler is used it is necessary to use a reference colour (ROX) for the recognition of the reaction tubes (capillaries) by the instrument.** The final concentration in the reaction volume of 10 µl must be 120 nM. It is recommended to replace the volume of Aqua dest. (5 µl) with the respective pre-dilution of ROX (240 nM).

If a premix of Q Primermix, Plex Mix and Aqua dest. is prepared for more than one sample please allow for a reasonable additional amount for pipetting losses.

If a **negative control (NTC)** should be performed prepare a PCR reaction with Aqua dest. instead of DNA.

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. Start the PCR program with the following parameters:

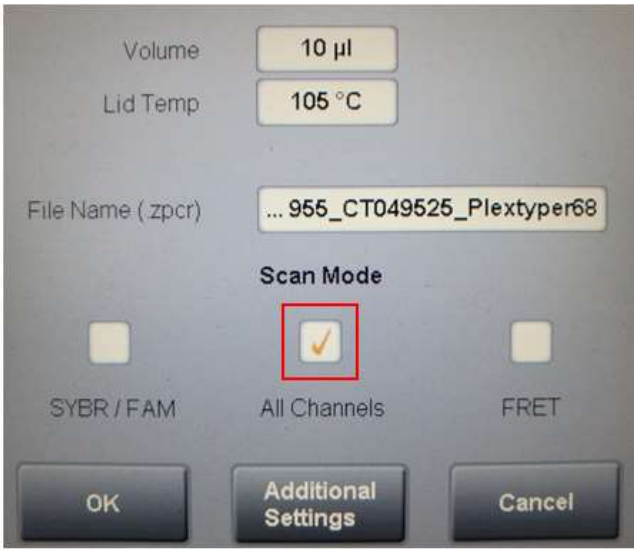
Step	Time [s]	Temperature [°C]	Ramp rate [°C/s]	Plate read	Cycles
Initial activation	120	96	2,5	-	1
Denaturation	5	98	2,5	-	13
Annealing + Extension	25	68	2,2	-	
Denaturation	5	98	2,5	-	37
Annealing + Extension	25	68	-*	yes	

* use the default ramp rate of the CFX96™ Real-Time PCR Detection System

~~The following realtime cyclers have been validated for the **FastQ B*27** kit:~~

~~Biorad: CFX96™ Real-Time PCR Detection System: use default settings~~

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.



Recommendation for tested, bot not validated cyclers:

- If the LightCycler® 480 II system is used a “colour compensation” has to be done (provided by BAG Diagnostics).
- If the Rotor-Gene Q cycler is used, the reaction volume must be set 20 µl in the cycler settings. The function "Use noise slop correction" can be used for data analysis.

6.4 Interpretation of results

All tests with 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. The red channel (CAL Fluor RED 610) gives positive signals with the independently detected B*27:06 and B*27:09 alleles.

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, *27:14, *27:15, *27:17, *27:19:01:01, *27:20, *27:24, *27:27	B*27:02:01:02-*27:02:01:05, *27:04:02-*27:04:06, *27:05:02:02- *27:05:02:20, *27:05:04-*27:05:46, *27:06:01:02, *27:07:02-*27:07:06, *27:11, *27:13, , *27:19:01:02, *27:21, *27:25, *27:26, *27:28 , *27:30-*27:74, *27:76, *27:79-*27:84, *27:86-*27:91, *27:93 -*27:118, *27:120-*27:128, *27:130-*27:152, *27:154-*27:156, *27:158-*27:188, *27:190-*27:203, *27:205-*27:221 / B*44:97

Fluorophor	Common	Well documented	Rare
CAL Fluor Red 610 (B*27:06, *27:09 positiv)	B*27:06:01:01	B*27:09	B*27:06:01:02, *27:41, *27:91, *27:106, *27:136, *27:154, *27:192, *27:208 / B*15:129, B*15:395 / B*18:02:01, B*18:179

(IMGT Database 3.38.0).

The amplification signals for the negative controls (B*27 negative) should be outside the defined Cq values for both channels. A negative control 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.

The following signals are rated as positive:

	Fluorophor	Cq-Value	Wave lenght in nm
Internal positive control	FAM	≤ 20	Excitation: 495 Emission: 520
B*27 positive	CAL Fluor Orange 560 (HEX)	≤ 25	Excitation: 538 Emission: 559
B*27:06 positive B*27:09 positive	CAL Fluor Red 610 (RED)	≤ 20	Excitation: 590 Emission: 610

Note: If the “Auto Calculated Baseline Threshold” is used for interpretation on the CFX96 Touch™ Real-Time PCR Detection System small fluctuations in the fluorescence signals may be regarded as positive signals by the software and a Cq is given. Please check the sigmoid shape of the curves and adjust the colour specific threshold during interpretation.

7. WARNINGS AND PRECAUTIONS

The **FastQ B*27** kit 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 extraction of DNA, 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**.

8. SPECIFIC PERFORMANCE CHARACTERISTICS

The combination of primers and probes ensures a reliable identification of the B*27 alleles specified in chapter 6.4. The accuracy and reproducibility of the specificity of the test kit is verified for each lot with pre-typed reference samples.

For the FastQ B*27 kit performance evaluation studies with a total of 95 pre-typed DNA samples were performed. The results from the study 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).

DNA samples	Internal study total	Percentage concordance [%]
B27 negative	84	100
B27 positive	11	100
Total	**95**	**100**

Summary of the internal study results with percentage concordance to the reference typing and detection of B*27

9. LIMITATIONS OF THE METHOD

Because of the high susceptibility of the RT-PCR method for cross contaminations special care should be taken during DNA isolation. Validation tests in the course of the performance evaluation study of the FastQ B*27 kit have shown that a variation of the amount of DNA used for the amplification between 2 ng and 50 ng do not have a significant influence on the detection of the B*27 alleles.
Extreme care should be taken to prevent contamination of the kit reagents and other laboratory materials and equipment with amplicons or DNA. Regular wipe tests (e.g. BAG Wipe Test, REF 7091) and negative controls with Aqua dest with each assay are strongly recommended.
In the negative control with Aqua dest. there must not be any fluorescent signal (Cq > N.A.). In the case of signal development in the negative control (FAM channel) the PCR working place has to be decontaminated and the reagents have to be exchanged if necessary.

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

10. INTERNAL QUALITY CONTROL

Internal quality control of new lots of the FastQ B*27 kit can be performed using a combination of DNA samples with known HLA type. An internal positive control for successful amplification is contained in the Q Primermix. Negative controls to detect possible contaminations are recommended. Use a PCR reaction without DNA (NTC) for this purpose.

11. 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.
	Wrong amplification parameters.	Check PCR program and ramp rate.
	Contaminated or degraded DNA.	Check DNA concentration and quality. Check DNA on a gel. Repeat DNA isolation.
	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 in the negative control	Repeat the negative control. Decontaminate the workplace.

12. TRADEMARKS USED IN THIS DOCUMENT/PRODUCT

TaqMan® is a trademark of Roche Molecular Systems Inc.

13. EXPLANATION OF SYMBOLS USED ON THE LABELS

	Sufficient for n tests
	Storage temperature / Lower limit of temperature
	Use by
	Consult instructions for use
	Manufacturer
CONT	Content, contains
GENOTYPING	Intended use: Typing of human genetic markers that are associated with diseases or pharmacogenetic reactions
IFU	Instructions for use
IVD	For in vitro diagnostic use
LOT	Batch code
Q PRIMERMIX | B27	Primermix for typing HLA-B*27 with the FastQ B*27 kit
PLEX MIX	Mastermix for RT-PCR
REF	Catalogue number

14. LITERATURE

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