EN

# 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**

IVD

**REF 728201 FastQ® B*27 direct** CE

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## 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 direct 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 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~~, packaged separately*, do not freeze!~~
- **Instructions for use**

~~*) When ordering a FastQ B*27 direct kit the Blood Booster, REF 728209 is supplied, too.~~

### 4.2 Additionally required reagents and devices

- Real-Time PCR-Cycler (Biorad CFX96™ and matching reaction tubes)
- Aqua dest.
- Piston pipettes (0,5 – 1000 µl) and tips

### 4.3 Validated cyclers and reaction tubes

| Cycler | RT-PCR reaction tubes | RT-PCR closing system |
|---|---|---|
| CFX96™ Real-Time PCR Detection System Comp. Bio-Rad | FrameStar® Break-A-Way PCR Plate, 96 white wells, black frame, Product No. 4ti-1201 Comp. 4titude / Brooks Life Sciences | Crystal Strips, Product No. 4ti-0755 Comp. 4titude / Brooks Life Sciences<br><br>qPCRSeal (Optically clear adhesive film) Product No. 4ti-0560 Comp. 4titude / Brooks 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 Blood Booster is shipped at ambient temperature and stored upon receipt at 2…8°C – **the Blood Booster should not be frozen.**~~ 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.

## 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 Sample preparation

EDTA whole blood or buffy coat has to be used as sample material. 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 should be used.

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 µl** Sample material (diluted 1:50 in A.dest)
**1 µl** Aqua dest.

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

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

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.

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

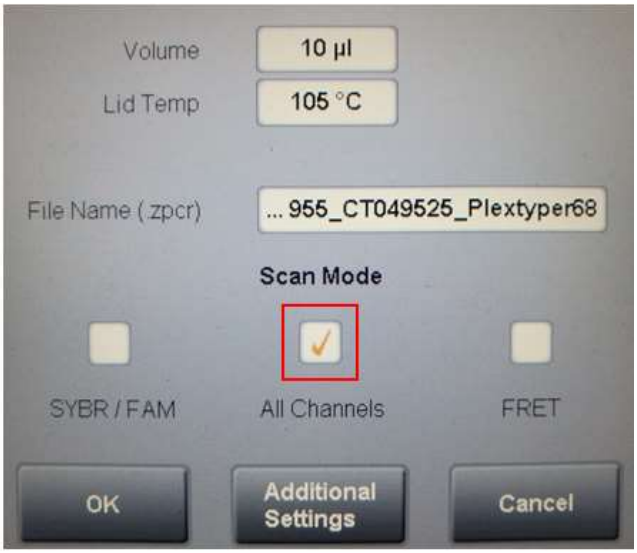
Close the reaction tubes and briefly spin down the liquid. Ensure that no bubbles are present in the wells. If bubbles are observed, gently tap assay on the bench to remove the bubbles.

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 | - | 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™ 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.




### 6.4 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 the negative controls (known B*27 negative samples) should 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 amplification control (IAC) | 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 CFX 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).

### 6.5 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, *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 |

IMGT Database 3.38.0

* Common and well documented Allele by CWD 2.0.0 catalogue(3)

## 7. 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**.

## 8. SPECIFIC PERFORMANCE CHARACTERISTICS

The combination of primers and probes ensures a reliable identification of the B*27 alleles specified in chapter 6.5. 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 direct** kit performance evaluation studies with a total of 120 pre-typed blood samples were performed to define the diagnostic sensitivity and specificity of the Q Primermix B27-d in combination with the Q Mastermix. The results from the studies 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 | Percentage concordance [%] |
|---|---|---|
| B27 negative | 101 | 100 |
| B27 positive | 19 | 100 |
| **Total** | **120** | **100** |

Table: Summary of the internal and external study results for the Q Primermix B27-d with percentage concordance to the reference typing and detection of 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.

## 9. 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 1:100 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 thoroughly 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. 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 (green channel / FAM) 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 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. Negative controls to detect possible contaminations are recommended. Use a PCR reaction without sample material (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<br>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<br>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<br>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<br>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<br>Decontaminate the workplace |

## 12. TRADEMARKS USED IN THIS DOCUMENT/PRODUCT

TaqMan® is a trademark of Roche Molecular Systems Inc.

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

## 13. EXPLANATION OF SYMBOLS USED ON THE LABELS

| | |
|---|---|
|  | Sufficient for n tests |
|  | Storage temperature / Lower limit of temperature |
|  | Storage temperature / Temperature limitation |
|  | Use by |
|  | Consult instructions for use |
|  | Manufacturer |
| BLOOD BOOST | Bood Booster, reagent for RT-PCR kits<br>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 |
| IFU | Instructions for use |
| IVD | For in vitro diagnostic use |
| LOT | Batch code |
| Q MASTERMIX | Mastermix for RT-PCR kits<br>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 |

## 14. 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

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