Instructions for Use

HISTO TYPE B*27 Q

Test kit for tissue typing of HLA alleles on a molecular genetic basis

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

REF **728200** **HISTO TYPE B*27 Q**

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1. PRODUCT DESCRIPTION

The HISTO TYPE B*27 Q 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-B27 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 **HISTO TYPE B*27 Q 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).

2. TEST PRINCIPLE

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 specially developed for the selective amplification of 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.

3. MATERIAL

3.1 Contents of the HISTO TYPE B*27 Q kit

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

3.2 Additionally required reagents and devices

- Reagents for DNA isolation (validated DNA isolation kits see 5.2)
- Real-Time PCR-Cycler (validated cycler see 3.3)
- RT-PCR reaction tubes with caps or foils (validated products see 3.3)
- Aqua dest.
- Piston pipettes (0,5 – 1000 µl) and tips

3.3 Validated cyclers and reaction tubes

Cycler	Software Version	RT-PCR reaction tubes	RT-PCR closing system
CFX96™ Real-Time PCR Detection System, Comp. Bio-Rad	3.1.1517.0823.	FrameStar® Break-A-Way PCR Plate, 96 clear wells, clear frame, Product No. 4ti-1200/C Comp. 4titude	4titude Crystal Strips, Product No. 4ti-0755 Comp. 4titude
		Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white #HSP9655 Comp. Bio-Rad	0.2 ml Flat PCR Tube 8-Cap Strips, optical, ultraclear #TCS0803 Comp. Bio-Rad
LightCycler® 480 System, Comp. Roche	LCS480 1.5.0.39	LightCycler® 480 Multiwell Plate 96, white, Product No. 04729692001 Comp. Roche	LightCycler® 480 Sealing Foil, Product No. 04729757001 Comp. Roche

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

4. STORAGE AND STABILITY

The kits are shipped with ice packs. 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 has no detrimental effects on the quality of the kit.

5. TEST PROCEDURE

5.1 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.

5.2 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)
- EXTRA-GENE I (salting out method)

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

A DNA concentration of 10-150 ng/µl is required to perform the HISTO TYPE B*27 Q 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.

5.3 Amplification

Reaction tubes recommended by the manufacturer of the realtime cycler or the materials recommended in chapter 3.3 should be used.

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

2 µl Q Primermix
2 µl Q Mastermix
1 µl Sample DNA (10-150 ng/µl)
5 µl Aqua dest.

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

If a premix of Q Primermix, Q Mastermix 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	Temperature	No. of cycles
Initial activation	10 min	96°C	1 cycle
Denaturation	20 sec	96°C	40 cycles
Annealing + Extension	40 sec + reading	64°C	

The following realtime cyclers have been validated for the HISTO TYPE B*27 Q kit:
Biorad: CFX96™ Real-Time PCR Detection System
Roche: LightCycler® 480 System

Special Note: If the LightCycler® 480 system is used the ramp rate has to be adjusted to 4.4°C/s for the denaturation and to 2.2°C/s for the annealing + extension step. Additionally a “color compensation” has to be done. With the CFX96™ Real-Time PCR Detection System the default settings should be used.
If other realtime cyclers are used they have to be validated by the user.

5.4 Interpretation of results

All tests with human gDNA must show a fluorescence signal in the yellow channel with the internal control. HLA B*27 positive samples show a positive signal in the green channel. The red channel gives positive signals with the independently detected B*27:06 and B*27:09 alleles.

Channel	Specificity
Green (B*27 positive)	B*27:01-17,19-21,24-28,30-47,76-84,86-156,158-164
Red (B*27:06, *27:09 positive)	B*27:06,09 #

The following alleles can not be excluded : B*27:91,106,136,154. This alleles are extremly rare and no CWD-alleles.

The amplification signals for the negative controls (B*27 negative) should be outside the defined Cq values for both channels (green and red). A negative control with Aqua dest. should not show any fluorescent signal during the complete Q-PCR run and repesents a contamination control.

The following signals are rated as positive:

	Channel	Pre-defined threshold	Cq-Level	LOD-Cq	Wave lenght in nm
Internal positive control	Yellow (VIC)	15	21	29	Excitation: 538 Emission: 554
B*27 positive	Green (FAM)	50	27	35	Excitation: 495 Emission: 520
B*27:06 positive B*27:09 positive	Red (Texas Red)	50	23	31	Excitation: 597 Emission: 616

Special Note: The pre-defined threshold should only be chosen for the combination of a CFX96 cycler with clear PCR reaction tubes. If only white PCR reaction tubes are used, the automatic threshold calculation of the respective software should be applied.
Cq-level is the PCR cycle that shows a positive detection against the background.
LOD-Cq is the latest PCR cycle that can be correctly rated as a positive detection against the background.

6. WARNINGS AND PRECAUTIONS

HISTO TYPE B*27 Q 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-healthcare.com.

7. SPECIFIC PERFORMANCE CHARACTERISTICS

The combination of primers and probes ensures a reliable identification of the B*27 alleles specified in chapter 5.4. The accuracy and reproducibility of the specificity of the test kit is verified for each lot with pre-typed reference samples.

For the HISTO TYPE B*27 Q kit performance evaluation studies with a total of 126 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 (amongs others serology, SSO, SSP) and/or sequencing. No discrepancies in the detection of the B*27 feature have been observed (100% concordance).

DNA samples	Total number (internal and external study)	Internal study total	External study total	Percentage concordance [%]
B27 negative	101	74	27	100
B27 positive	25	21	4	100
Total	**126**	**95**	**31**	**100**

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

8. LIMITATIONS OF THE METHOD

Because of the high susceptibility of the Q-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 HISTO TYPE B*27 Q kit have shown that a variation of the amount of DNA used for the amplification between 10 ng and 150 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 (yellow 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.

9. INTERNAL QUALITY CONTROL

Internal quality control of new lots of the HISTO TYPE B*27 Q kit can be performed using a combination of DNA samples with known HLA type. A 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.

10. 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 para-meters.	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 qPCR plastic ware.	Use compatible and high quality plastic ware (see chapter 3.3).
	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.

11. TRADEMARKS USED IN THIS DOCUMENT/PRODUCT

TaqMan® is a trademark of Roche Molecular Systems Inc.

12 EXPLANATION OF SYMBOLS USED ON THE LABELS

Symbol	Explanation
Σ	Sufficient for n tests
	Storage temperature / Lower limit of temperature
	Use by
	Consult instructions for use
	Manufacturer
HLA TYPING	Intended use: HLA typing
IFU	Instructions for use
IVD	For in vitro diagnostic use
LOT	Batch code
Q Primermix | B27	Primermix for typing HLA-B*27 with the HISTO TYPE B*27 Q kit
Q Mastermix	Mastermix for the HISTO TYPE B*27 Q kit
REF	Catalogue number

13. LITERATURE

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