



1. Intended Use	Code: KT0003
The test kit MutaGEL® HLA B27 allows for the analysis of DNA in respect to detection of the HLA B27- alleles present in a human probe. <i>For research use only.</i>	

2. Introduction
<p>Patients with ankylosing spondylitis (Morbus Bechterew = M. B.) have cell surface molecules of the HLA- B27 group in 90 % of the cases, whereas the normal abundance is only up to 10 % positives. Absence of a B27- allele in a person can therefore diminish the probability of a M.B. diagnosis in the internistically demanding diagnosis of spondylarthropathies (especially in young people). Additionally segregation between certain "disease coupled" B27- types has been worked out by the modern molecular medical research (actually there are 35 human B27- alleles known differing in amino acid changes in their protein sequence – an "update" for MutaGEL® HLA B27 is done periodical). Epidemiological relevant are only the alleles *2701 - *2710; all other HLA B27- types are described till now only in single cases.</p> <p>That means, only some of the B27- allele types are abundant: Types *2701, -02, -03, -04, -05, -07, -08 and -10 are associated with the chronic inflammatory rheumatic disease M. B. and population studies for them are already available. The types *2706, -09, -11 and -18 are not associated with M. B., most of the others having been reported only once. This variability characterizes the frame of the diagnostic demand and the possibility of occurrence of further new sequence findings must always be kept in mind.</p>

3. Principle of the Test
<p>The PCR kit MutaGel® HLA-B27 contains reagents for two types of DNA amplification: Test A is a "surveying" approach, which can detect all known sequences of the HLA B27 group contained in a human probe (= types *2701 - *2735). On the other hand Test B gives positive results with nearly all known M. B. associated B27- types (especially again alleles *2705 and *2702, who together asymptotically add to 100 % in Middle European B27 carriers and M.B. patients as well as alleles *2703 and *2704 from M.B. cases of Africa and Asia). Furthermore, in Test B the presumably protective *2706 and famous *2709 are excluded from its amplification.</p> <p>Therefore Test A should be done for the general HLA B27 search and Test B for the analysis of existence of HLA B27 disease associated alleles (of course the alleles seen by Test B are all detected by Test A also and the direct comparison allows conclusion for protective alleles).</p>

4. Material Supplied (24 determinations)
<ul style="list-style-type: none"> ▪ PCR-Mix 1 HLA-B27 1 x 550 µl (green) reagents for HLA-B27 specific amplifications (hotstart PCR); "wide" ▪ PCR-Mix 2 ankylosing spondylitis (M.B.) 1 x 550 µl (lilac) reagents for M.Bechterew associated amplifications (hotstart PCR); "close" ▪ HLA B27 Positive Control DNA 1 x 30 µl (red) solution with DNA amplicons from Test A and Test B positive PCRs ▪ PCR water 1 x 200 µl (white) deionized water (molecular biological grade)

5. Materials Required but not Supplied
<p>Reagents and Instruments:</p> <ul style="list-style-type: none"> ▪ DNA extraction kit (f. e. "BLOOD MINIPREP" kit; Code: KBR3005) ▪ Reagents for gel electrophoresis ▪ Thermal cycler ▪ Pipettes (0.5 - 200 µl) and sterile pipette tips ▪ Sterile micro tube (for master mix preparation) ▪ Instruments for gel electrophoresis

6. Storage and Stability
<p>Store at < -18°C. The reagents are stable in the unopened micro tubes until the expiration date indicated (see print on the package). Do not thaw out the content of the "HLA B27 Positive Control DNA" for more than two times. If necessary, make suitable aliquots.</p> <p><i>Before use:</i> Spin tubes briefly before opening (contents may become dispersed during shipment).</p>

7. Warning and Precautions
<ul style="list-style-type: none"> ▪ For research use only. ▪ PCR tests should be performed only by skilled persons considering GLP (Good Laboratory Practice) guidelines. ▪ Don't use the kit after its expiration date. ▪ After usage, dispose all reagents and test components included in the kit in conventional garbage. ▪ PCR technology is extremely sensitive. The amplification of a single DNA molecule generates million identical copies. Therefore set up three separate working areas for a) sample preparation, b) PCR reagent preparation and c) DNA detection. For each working area a different set of pipettes should be reserved. ▪ Wear separate coats and gloves in each working area. ▪ Use sterile filter tips for pipetting and use special PCR pipettes for aerosol free pipetting. ▪ Routinely decontaminate your pipettes and the laboratory benches. ▪ Avoid aerosols.



Procedure

The complete procedure is divided in three steps:

1. Sample preparation.
2. Amplification with two primer sets specific for the HLA B27- alleles and for Morbus Bechterew associated HLA B27- alleles (Tests A and B).
3. Analysis of genotype by gelelectrophoretic separation of the amplified DNA-products.

8. Sample Preparation

- For template use total genomic DNA which can be extracted (f. e. from 200 µl whole blood) using a commercial available DNA isolation kit according to the manufacturers instruction.
- Start immediately with the amplification procedure or store the extracted DNA at ≤ -20°C.

9. Amplification

- Every set of amplifications should include a positive as well as a negative control.
- For each sample, positive control and negative control, prepare the following Master Mix (multiply the volumes necessary for each reaction with the number N of reactions and add 10 % more volume):

PCR -reagents	Reaction Volume: 25 µl	Master Mix - Volume
PCR master mix	20 µl	20 µl x (N + 10%)
<ul style="list-style-type: none"> ▪ Add 20 µl of the prepared master mix to the necessary PCR tubes. ▪ Samples: add 5 µl each of the extracted gDNA (about 20 ng/µl) to the corresponding PCR tube. ▪ Positive control: add 5 µl of HLA B27 positive control DNA to the Master Mix of the positive reference PCR tube. ▪ Negative control: add 5 µl of PCR water to the Master Mix of the negative reference PCR tube. ▪ transfer the micro tubes into the thermal cycler. ▪ perform the following amplification protocol: 		
Initial hold:	95°C for 10 min	
35 cycles:	95°C for 30 sec / 58°C for 30 sec / 72°C for 1 min	
Strand completion:	74°C for 10 min	
Final hold:	10°C	

10. Detection of the amplified DNA and Interpretation of the Results

- Carry out gel electrophoresis in **2%** agarose (or 10 % polyacrylamid) for about **120 Vh** (f.e. 80 min at 90 volts) in 1x TBE-buffer: Mix about **15 µl** of each amplified material with **4 µl** loading buffer (f.e. KAN01070) and load the sample to the gel. The length of the detected DNA fragments can be equalized with a suitable molecular weight standard (f. e. KBR311005). The separated DNA is coloured by ethidium bromide or SybrGreen (5 µg/ml) for 5 - 10 min and visualised under UV-light (312 nm).
- The PCR results for positive samples (and positive control) in an allele-specific DNA-fragment of **100 bp** length in **Test A** and of **105 bp** in **Test B** as well as an internal control fragment of about **400 bp** each. (*Please consider:* if HLA B*27 alleles are present, the internal control can be weaker).
- In any case the negative controls must be negative for any amplification product.
- Case 1: **Test A** and **Test B** are both **positive**: the probe contains one or two B27- alleles associated with occurrence of Morbus Bechterew (ankylosing spondylitis).
- Case 2: **Test A** is **positive** and **Test B** is **negative**: the probe contains one or two B27- alleles not associated with Morbus Bechterew (ankylosing spondylitis) consequently *2706 or *2709 (or a rare new sequence variant, which should be sequence when the clinical diagnosis of ankylosing spondylitis is obvious).
- (Case 3: **Test A negative** and **Test B positive**: not possible, technical failure.)

11. Restrictions

In very rare cases (1 % of populations from Iran, Mongolia, people from Oman) the Test A - PCR can produce false positives. This phenomenon is in general corrected for disease associated B27 types by Test B, which is not sensitive for the crossreactivity of this non HLA-B27 group. For such cases DNA sequence analysis is useful, which is available from the distributor.

Also Test B can produce false positives, because alleles of single case isolates like *2712 and *2718 reported from sane persons could prove protective in the future. This is on the other hand unlikely, because the Test B-PCR bases on detection of the disease associated amino acid 116 Asp and then the "protectivity" is more likely to be a result of B27s restricted disease penetrance.

The single surely disease associated type, which can not be detected by test B is HLA-B*2707 due to its amino acid tyrosine at position 116. This allele exists only in some populations of the world and is very rare in Middle European patients. If in general a discrepancy between the clinical diagnosis and the test results would occur, the person responsible is encouraged to ask **Immundiagnostik AG** for a **sequence analysis**.

If there are no positive control DNA fragments present, the chosen PCR conditions have to be corrected. The sample must be tested a second time or the complete analysis must be repeated with freshly isolated DNA.