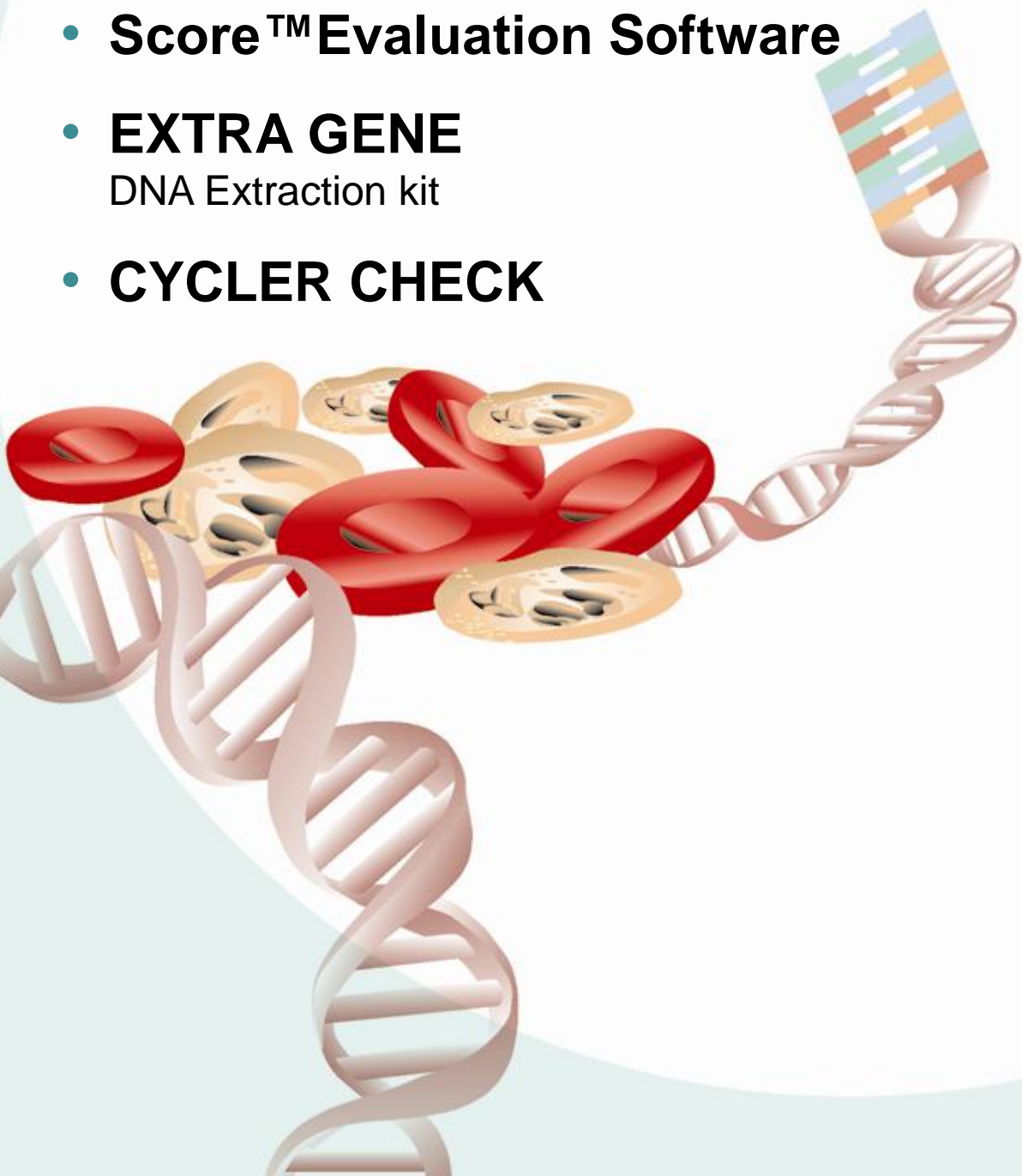


# HLA-SSP

- **HISTO TYPE**  
SSP typing kits for HLA Class I + II **low resolution**  
SSP typing kits for HLA Class II **high resolution**
- **KIR Type**
- **Score™ Evaluation Software**
- **EXTRA GENE**  
DNA Extraction kit
- **CYCLER CHECK**



## HISTO TYPE SSP-testkits for typing of HLA-Class I+II antigens (low resolution)

Product	Unit Size	REF	Required Taq
<b>HISTO TYPE A low</b> 24 PCR Mixes + Contamination Control	20 Tests	70721	44 µl
<b>HISTO TYPE B low</b> 48 PCR Mixes + Contamination Control	20 Tests	70731	84 µl
<b>HISTO TYPE C low</b> 23 PCR Mixes + Contamination Control	20 Tests	70741	44 µl
<b>HISTO TYPE DR low</b> 24 PCR Mixes + Contamination Control	20 Tests	70751	44 µl
<b>HISTO TYPE DRB3/4/5</b> 3 PCR Mixes + Contamination Control	20 Tests	70771	8 µl
<b>HISTO TYPE DR mini</b> 8 PCR Mixes + Contamination Control	20 Tests	70761	16 µl
<b>HISTO TYPE DQB low</b> 8 PCR Mixes + Contamination Control	20 Tests	70891	16 µl
<b>HISTO TYPE ABC</b> 95 PCR Mixes + Contamination Control	20 Tests	7102	164 µl
<b>HISTO TYPE ABDR</b> 96 PCR Mixes + Contamination Control	20 Tests	7098	164 µl
<b>HISTO TYPE ABDR 384</b> 4x96 PCR Mixes + Contamination Control	100 Tests	7098CN	380 µl
<b>HISTO TYPE DR/DQB</b> 32 PCR Mixes + Contamination Control	20 Tests	7103	58 µl

## HISTO TYPE SSP-testkits for typing of HLA-Class II antigens (high resolution)

Product	Unit Size	REF	Required Taq
<b>HISTO TYPE DRB1*01</b> 8 PCR Mixes + Contamination Control	12 Tests	70762	12 µl
<b>HISTO TYPE DRB1*03</b> 17 PCR Mixes + Contamination Control	12 Tests	70792	22 µl
<b>HISTO TYPE DRB1*04</b> 24 PCR Mixes + Contamination Control	12 Tests	70802	26 µl
<b>HISTO TYPE DRB1*07/09</b> 8 PCR Mixes + Contamination Control	12 Tests	70822	12 µl
<b>HISTO TYPE DRB1*08/12</b> 20 PCR Mixes + Contamination Control	12 Tests	70852	24 µl
<b>HISTO TYPE DRB1*11</b> 20 PCR Mixes + Contamination Control	12 Tests	70812	24 µl
<b>HISTO TYPE DRB1*13</b> 27 PCR Mixes + Contamination Control	12 Tests	70832	30 µl
<b>HISTO TYPE DRB1*14</b> 27 PCR Mixes + Contamination Control	12 Tests	70842	32 µl
<b>HISTO TYPE DRB1*15</b> 9 PCR Mixes + Contamination Control	12 Tests	70772	14 µl
<b>HISTO TYPE DRB1*16</b> 7 PCR Mixes + Contamination Control	12 Tests	70782	12 µl
<b>HISTO TYPE DQB high</b> 47 PCR Mixes + Contamination Control	10 Tests	709010	46 µl
<b>HISTO TYPE DQB1*02/04</b> 8 PCR Mixes + Contamination Control	12 Tests	70902	12 µl
<b>HISTO TYPE DQB1*03</b> 20 PCR Mixes + Contamination Control	12 Tests	70903	24 µl
<b>HISTO TYPE DQB1*05</b> 5 PCR Mixes + Contamination Control	12 Tests	70904	10 µl
<b>HISTO TYPE DQB1*06</b> 21 PCR Mixes + Contamination Control	12 Tests	70905	26 µl

## HISTO TYPE SSP-Testkits for Disease Associations

Product	Unit Size	REF	Required Taq
<b>HISTO TYPE B27</b> 1 PCR Mix	48 Tests	7070	5 µl
	96 Tests	7071	10 µl
<b>HISTO TYPE B27 high</b> 16 PCR Mixes + Contamination Control	12 Tests	70710	20 µl
<b>HISTO TYPE B57</b> 7 PCR Mixes + Contamination Control	20 Tests	70715	16 µl
<b>HISTO TYPE Celiac Disease</b> 23 PCR Mixes + Contamination Control	20 Tests	70941	44 µl

## HISTO TYPE Null Allele Kits SSP-testkits for typing of HLA-Class I antigens (NMDP Policy for confirmatory typing)

Product	Unit Size	REF	Required Taq
<b>HISTO TYPE Null A*2409N</b> 1 PCR Mix	24 Tests	70862	3 µl
<b>HISTO TYPE Null B*5111N</b> 1 PCR Mix	24 Tests	70872	3 µl
<b>HISTO TYPE Null Cw*0409N</b> 1 PCR Mix	24 Tests	70882	3 µl

## Additional SSP Testkits

<b>KIR TYPE</b> 20 PCR mixes + positive/negative control	10 tests	7105	19 µl
<b>EPITOP TYPE</b> 5 PCR mixes + Contamination Control	10 tests	7106	5 µl

## Accessories

<b>EXTRA GENE I</b> Kit for extraction of genomic DNA (salting out method)	50 extractions	7059	
<b>HISTO TAQ</b> Taq polymerase for HISTO TYPE kits	50 µl	70975	
<b>DNA-Length Standard</b> (φX174/Hae III;72-1.353bp)	0,5 ml	7097	
<b>Wipe Test</b> Contamination Control for HLA-Class I+II	40 reactions	7091	
<b>CYCLER CHECK</b> Kit for evaluation of temperature uniformity in thermal cyclers	10 tests 4 tests	7104 71044	
<b>SCORE™ Evaluation Software</b>	1	7056	

## HISTO TYPE SSP HLA Typing

### Low resolution

The HISTO TYPE SSP product line offers reliable and convenient low resolution HLA typing for HLA-A, B, C, DRB and DQB1.

#### Your advantages:

- All kits work with the same protocol and can, therefore, be done simultaneously.
- The primers are selected to provide optimal resolution with a minimum of ambiguities
- PCR buffer and PCR program are optimized for high stringency, reducing unspecific reactions

### High resolution

HISTO TYPE SSP enables high resolution typing of HLA-DRB1 and DQB1 with the same protocol as the low resolution kits. The kits are designed to give an unambiguous result for frequent alleles (> 0,5 % allele frequency).

#### Your advantages:

- More than 95% of your samples will be typed unambiguously
  - With less PCR mixes
  - With less Taq-Polymerase
  - With higher throughput
- Well-priced sequencing service of for ambiguous results usually including rare alleles

Easy interpretation and data management with the SCORE™ Evaluation Software

## Short Instructions

### HLA typing using HISTO TYPE SSP test kits

1. DNA isolation (e.g. with EXTRA GENE I)
2. Prepare PCR mix using the following reagents:
  - DNA (50 ng/ Mix)
  - 10x PCR-Buffer
  - Aqua dest.
  - Taq Polymerase
3. Add 10 µl of sample mix into each pre-aliquoted reaction mix and close well with caps or foil
4. Start the PCR program:

Program Step	Temperature	Time	No. of Cycles
First Denaturation	96°C	5 min.	1 cycle
Denaturation	96°C	20 sec.	5 cycles
Annealing + Extension	68°C	60 sec.	
Denaturation	96°C	20 sec.	10 cycles
Annealing	64°C	50 sec.	
Extension	72°C	45 sec.	
Denaturation	96°C	20 sec.	15 cycles
Annealing	61°C	50 sec.	
Extension	72°C	45 sec.	
Final Extension	72°C	5 min.	1 cycle

5. Detection with gel electrophoresis and ethidium bromide staining
6. Photo documentation and interpretation

## SCORE™ Evaluation Software

The HELMBERG SCORE™ Software was developed by Dr. W. Helmberg („Virtual DNA analysis - a new tool for combination and standardised evaluation of SSO, SSP and sequencing-based typing results", Tissue Antigens ·1998, 51:587-592). The Windows-based interpretation software allows a „virtual HLA-DNA-Analysis" of the results generated with the BAG HISTO TYPE SSP test kits (low resolution and high resolution). Regular updates of the allele-database ensure HLA typing on the latest standards of the HLA - Nomenclature. Easy data input by virtual gels, extensive interpretations and various methods of documentation facilitate an accurate evaluation and management of the HLA-data.

### System requirements:

PC with Pentium 266 or higher, at least 32 MB RAM, CD-ROM  
Screen resolution at least 800 x 600  
Windows 95/98, Windows NT or higher

## HISTO TYPE Null Allele Kits

### NMDP Policy for confirmatory typing – effective June 1, 2008

“.... laboratories **are required** to test for the following three CWD null alleles where evidence exists that the individual being typed may carry alleles and/or haplotypes that have been associated with a specific null allele.”

Null allele	Alternative common allele	Associated alleles in haplotype	Location of polymorphism
A*2409N	A*24020101	B*40 or B*27	Exon 4
B*5111N	B*510101	A*02 or DRB1*04 or Cw*15	Exon4
Cw*0409N	Cw*04010101	B*4403	Exon 7

**BAG** offers kits for the detection of these CWD null alleles.

Each kit contains 24 tests (3 strips of 8 wells), 10x per buffer, per caps and instructions for use. The cycling parameters are the same as for all **HISTO TYPE** products.

Product	Unit Size	REF
HISTO TYPE Null A*2409N	24 tests	70862
HISTO TYPE Null B*5111N	24 tests	70872
HISTO TYPE Null Cw*0409N	24 tests	70882

# HISTO TYPE DR mini

## HLA SSP pre-typing kit before sequencing the DRB1 gene

Sequencing becomes more and more the method of choice for high resolution DR-typing. The most unambiguous results are obtained with sequencing PCR products after a group specific amplification.

With only 8 SSP PCR reactions the **HISTO TYPE DR mini** kit (REF 70761) allows a low resolution DR typing as it is usually needed for sequencing after group specific amplification.

The following allele groups can be separated:

**DRB1\*01**

**DRB1\*15/16**

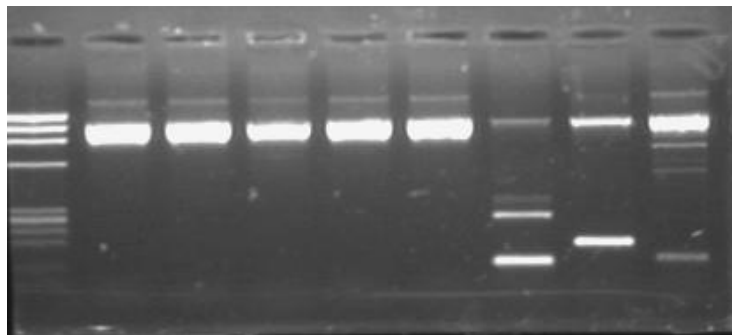
**DRB1\*04**

**DRB1\*07**

**DRB1\*10**

**DRB1\*12**

**DRB1\*03/08/11/13/14**



## KIR-TYPE / EPITOP TYPE

Natural killer cells (NK) and subpopulations of T-lymphocytes (CD8<sup>+</sup> memory phenotype) express inhibitory and activating Killer-cell *Immunoglobulin-like* receptors (KIRs), which recognize HLA class I molecules. Inhibitory KIRs have usually a higher affinity for MHC class I ligands and therefore co-ligation of both activating and inhibitory receptors results in a net negative signal followed by no activation of the NK cell. If the corresponding ligand for inhibitory receptors is missing or a higher number of activating receptors is recognizing their ligands the activating signal is dominating resulting in the activation of the NK cell and lysis of the target cell. Meanwhile a large number of studies have demonstrated that HLA/KIR disparity leads to donor versus recipient NK cell reactivity in bone marrow transplantation resulting in the reduction of Graft versus Host Disease (GvHD) and relapse (1). Furthermore defined KIR genotypes have been associated with autoimmune diseases (Psoriasis, Rheumatoid Arthritis) (2,3) and the risk of preeclampsia (4).

The **KIR-TYPE** Kit allows the genotyping of 14 KIR genes and 2 pseudogenes. The **EPITOP-TYPE** kit detects the alleles of KIR-HLA ligands HLA-Cw Asn<sup>80</sup>, HLA-Cw Lys<sup>40</sup>, HLA-B Bw4<sup>Threo</sup>, HLA B Bw4<sup>Iso</sup> and HLA-A Bw4. The detection of the single Kir genes / KIR HLA ligands is performed applying the PCR-SSP method (*PCR-sequence-specific primers*) Selected amplification primers allow the investigation of KIR genes at the genomic DNA level and the detection of their transcripts at the mRNA level. Pre-aliquoted PCR reactions with a volume of 10µl include internal amplification controls.

**Package contents KIR-TYPE (REF 7105): 20 PCR mixes, 1 positive control, 1 negative control, 10 tests**

**Package contents EPITOP-TYPE (REF 7106): 5 PCR mixes, 1 positive control, 1 negative control, 10 tests**

1. Ruggeri L, Capanni M, Mancusi A, Martelli MF, Velardi A. The impact of donor natural killer cell alloreactivity on allogeneic hematopoietic transplantation. *Transpl Immunol*. 2005 Aug;14(3-4):203-6. Review

2. Martin MP, Nelson G, Lee JH, Pellett F, Gao X, Wade J, Wilson MJ, Trowsdale J, Gladman D, Carrington M. Cutting edge: susceptibility to psoriatic arthritis: influence of activating killer Ig-like receptor genes in the absence of specific HLA-C alleles. *J Immunol*. 2002 Sep 15;169(6):2818-22.

3. Martin MP, Nelson G, Lee JH, Pellett F, Gao X, Wade J, Wilson MJ, Trowsdale J, Gladman D, Carrington M. Cutting edge: susceptibility to psoriatic arthritis: influence of activating killer Ig-like receptor genes in the absence of specific HLA-C alleles. *J Immunol*. 2002 Sep 15;169(6):2818-22.

4. Hiby SE, Walker JJ, O'shaughnessy KM, Redman CW, Carrington M, Trowsdale J, MoHett A. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *J Exp Med*. 2004 Oct 18;200(8):957-65.

## Short Instructions

### KIR-/EPITOP typing using HISTO TYPE SSP test kits

1. DNA isolation (e.g. with EXTRA GENE I)
2. Prepare PCR mix using the following reagents:
  - DNA (50 ng/ Mix)
  - 10x PCR-Buffer
  - Aqua dest.
  - Taq Polymerase
3. Add 10 µl of sample mix into each pre-aliquoted reaction mix and close well with caps or foil
4. Start the PCR program:

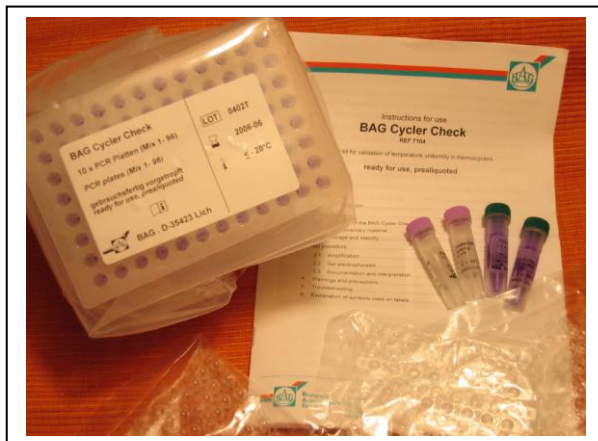
Program Step	Temperature	Time	No. of Cycles
First Denaturation	95°C	2 min.	1 cycle
Denaturation	95°C	15 sec.	5 cycles
Annealing + Extension	65°C	60 sec.	
Denaturation	95°C	15 sec.	10 cycles
Annealing	61°C	50 sec.	
Extension	72°C	30 sec.	

5. Detection with gel electrophoresis and ethidium bromide staining
6. Photo documentation and interpretation

## CYCLER CHECK

According to EFI (P2.1313) and ASHI guidelines thermal cyclers must regularly be tested for accuracy and reliability of specified temperatures.

Regular checks of thermal cyclers by the manufacturer are very expensive and cause disturbances in the laboratory due to the absence of instruments. The single-vessel-control involves a lot of work by testing all 96 wells for a complete check of the thermal cycler.

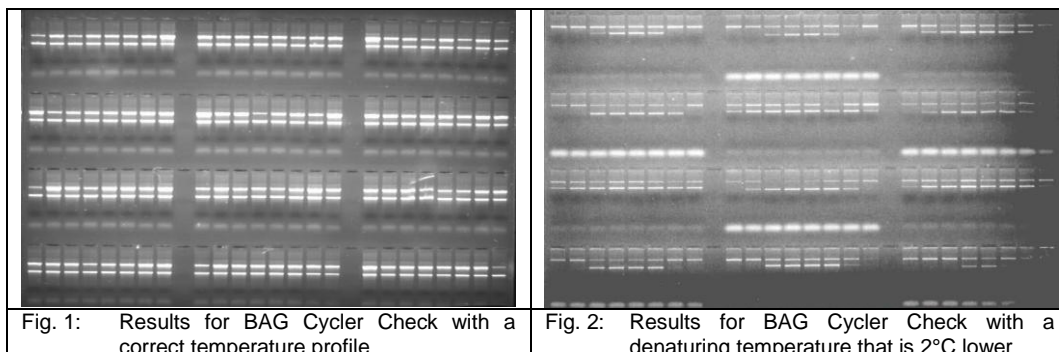


The **BAG-Cycler Check** enables a reliable and cost-effective check of the complete 96 well thermalblock. The **BAG-Cycler Check** contains a 96 well PCR plate with the same testmix in all positions. The reaction mix contains one primer pair for validation of the denaturing temperature (540 bp) and another primer pair for validation of the annealing temperature (1040 bp).

The two primer pairs are designed to yield optimal results under the same PCR conditions. Therefore, with a correct and uniform temperature profile there should be two bands in each of the 96 positions (Fig. 1).

Lower denaturing temperatures and higher annealing temperatures result in weaker reactions or the loss of bands in single positions or in all positions (Fig. 2).

A reliable check of the temperature uniformity for the complete thermalblock is achieved with only one PCR run.



**Package contents: positive control DNA, 96er PCR plates with prealiquoted reaction mix, 10x PCR buffer, PCR foil, instructions for use (REF 7104 = 10 tests / REF 71044 = 4 tests).**

## Wipe Test

According to EFI (L1.2200) and ASHI guidelines the laboratory areas, especially the Pre-PCR area (DNA extraction, PCR setup) must regularly be tested (every 2 month) for possible contaminations with amplicons or genomic DNA.

It is also recommended to check the laboratory working materials and single reagents (e.g. Taq Polymerase) to secure the quality of the laboratory and to avoid contaminations, which would lead to false positive reactions.



The **Wipe Test** is well suited for a reliable and fast check of contaminations with amplicons of the HLA I + II genes and genomic DNA.

The **Wipe Test** contains 5 x 8 well PCR strips, which are pre-aliquoted with the same test mix in every position.

The reaction mix contains a primer mixture for the detection of contaminations with amplicons of the HLA class I + II genes and genomic DNA.

**Package contents (REF 7091): 10x PCR buffer, 5x8 PCR stripes, PCR caps, instructions for use**

