

**EN**

# Instructions for use

## HISTO TYPE SSP Kits

**IVD**

Electronic instructions for use see [www.bag-diagnostics.com](http://www.bag-diagnostics.com)

Test kits for tissue typing of HLA alleles on a molecular genetic basis  
(Class I: HLA-A, B, C and Class II: HLA-DR, DQ)

ready to use prealiquoted

<b>REF</b> 70721	<b>HISTO TYPE A low</b>	
<b>REF</b> 70731	<b>HISTO TYPE B low</b>	
<b>REF</b> 70751	<b>HISTO TYPE DR low</b>	
<b>REF</b> 7098	<b>HISTO TYPE ABDR</b>	
<b>REF</b> 7102	<b>HISTO TYPE ABC</b>	
<b>REF</b> 7103	<b>HISTO TYPE DR/DQB</b>	<b>CE</b> 0123
<b>REF</b> 7070	<b>HISTO TYPE B27 low</b>	
<b>REF</b> 7071	<b>HISTO TYPE B27 low</b>	
<b>REF</b> 70941	<b>HISTO TYPE Celiac Disease</b>	
<b>REF</b> 70715	<b>HISTO TYPE B*57:01/B*51</b>	
<b>REF</b> 70716	<b>HISTO TYPE Narcolepsy</b>	
<b>REF</b> 70741	<b>HISTO TYPE C low</b>	<b>CE</b>
<b>REF</b> 70891	<b>HISTO TYPE DQB low</b>	
<b>REF</b> 709010	<b>HISTO TYPE DQB high</b>	

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**Version: 20/2020 / Issued: 2020-08** Changes to version 19/2019 are highlighted in yellow!

## 1. Product description

The **HISTO TYPE Kits** are used for HLA typing on a molecular genetic basis (information on testkits for typing of disease-associated HLA alleles see chapters 1.1, 1.2, 1.3 and 1.4).

The basic material for typing with **HISTO TYPE SSP kits** is purified DNA. The test procedure is done by using the Sequence Specific Primers (SSP) -PCR (see Fig. 1) [2, 3]. This method is based on the fact that primer extension and hence successful PCR relies on an exact match at the 3'-end of both primers. Therefore, only if the primers entirely match the target sequence is amplification obtained which is subsequently visualized by agarose gel electrophoresis.

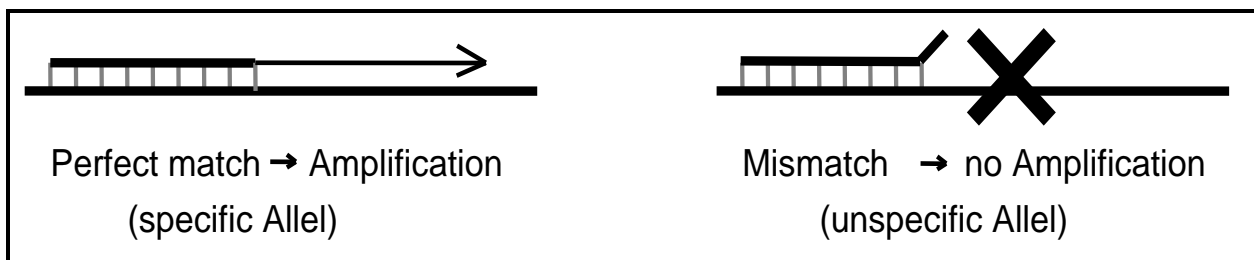


Fig. 1: Principle of SSP-PCR

The composition of the individual primer mixtures makes clear identification of the HLA types indicated in the respective evaluation diagrams possible. With each typing a certain number **prealiquoted** and **dried** reaction mixes including internal amplification control with a final volume of 10 µl are used.

### 1.1 Short background HISTO TYPE Celiac Disease

Celiac disease is an autoimmune reaction triggered by gluten which is an ingredient of different cereals. If not diagnosed early this leads to chronic inflammation and destruction of the small intestine. Celiac disease is strongly associated with the DQA1\*05:01-DQB1\*02:01 and DQA1\*03-DQB1\*03:02 haplotype. Additionally, DR3, DR7 and DR11 alleles can be used as genetic markers. [8-10]

### 1.2 Short background HISTO TYPE B\*57:01/B\*51

A treatment with antiretroviral drugs (e.g. for HIV therapy) containing the active substance Abacavir is permitted only, if the allele HLA B\*57:01 is excluded in the patient. This is due to a potential hypersensitivity reaction that is associated with this allele. [11-13]

Behcet's disease is a chronic vasculitis characterized by recurrent oral ulcers, genital ulcers, ocular and skin involvement, and other multisystemic features. Despite a worldwide distribute Behcet's disease clusters in an area from eastern Asia to the Mediterranean basin. HLA B\*51 is a strong risk factor for the disease and can be used as a diagnostic tool. [14]

### 1.3 Short background HISTO TYPE Narcolepsy

Narcolepsy is a sleep disorder with symptoms like excessive daytime somnolence, sleep paralysis or hallucinations. 98 % of the Caucasian narcolepsy patient has the DRB1\*15:01 – DQA1\*01:02 – DQB1\*06:02 haplotype. Therefore, HLA Typing is helpful to confirm or exclude a diagnosis. [15-17]

### 1.4 Short background HISTO TYPE B27

The association between HLA-B27 and the group of diseases summarized as seronegative arthritis (Bechterew's disease, Reiter's disease, reactive arthritis) is commonly used as part of the diagnostic procedure. A positive HLA-B27 result is associated with a very high disease risk (see Table 1) [18, 19]. Most notably, a confirmed HLA-B27 diagnostics result makes an important contribution to the therapy of the patient in unclear cases of suspected Bechterew's disease.

Disease	B27 Frequency in patients	Relative risk
Ankylosing spondylitis (Bechterew's disease)	90.2 %	91
Reiter's disease	78.8 %	37.6
Post-infection reactive arthritis	70.2 %	

Table 1: HLA-B27 Frequencies and risks.

## 2. Material

### 2.1 Contents of the HISTO TYPE SSP kits

- ◆ HISTO TYPE plates/strips for the HLA typing. The pre-dropped and dried reaction mixtures consist of allele specific primers, internal control primers (specific for the human G3PDH gene) and nucleotides. The first reaction mix is marked (please see mix-arrangement on page 7). In most of the HISTO TYPE products the contamination control is integrated on the first or the last position. It can be identified by a different colour (blue mix). The lot number is printed on each plate/ strip.
- ◆ PCR strips (á 8) contamination control with internal control primers and amplificate specific primers (not separately, if the contamination control is integrated in the test plate/strip and in the HISTO TYPE B27 low kit).
- ◆ 10x PCR buffer
- ◆ Strip-caps or PCR foil
- ◆ Information CD (contains instructions for use for HISTO TYPE and HISTO MATCH\*\*, specificity table\*, hit table\*, worksheet, list "Untested Primers", batch file for HISTO MATCH\*\* and SCORE\*, quality control certificate)

\*not for HISTO TYPE B27 low / \*\*not for HISTO TYPE B27 low and HISTO TYPE Narcolepsy

## 2.2 Requirements and supplementary material

- ◆ Happy Taq (REF 70976) (or another Taq Polymerase, validated with the HISTO TYPE kits by the user).

The Happy Taq is supplied free of charge when ordering a HISTO TYPE Kit.

### **Don't use a Hot-start Taq Polymerase please!**

- ◆ **BAG EXTRA-GENE I** Kit (REF 7059) for DNA extraction from blood / lymphocytes / leucocytes or material for other DNA extraction methods
- ◆ piston pipettes (0.5-250 µl)
- ◆ sterile tips with integrated filter
- ◆ DNA Cycler (list of the validated cyclers please see chapter 4.3)

## Devices and material for gel electrophoresis

- ◆ DNA agarose
- ◆ 0.5 x TBE buffer (45 mM of Tris base, 45 mM of boric acid, 0.5 mM of EDTA)
- ◆ Ethidium bromide (EtBr)
- ◆ submarine electrophoresis unit
- ◆ power supply (200 - 300 V, 200 mA)
- ◆ DNA-length standard (REF 7097)

## Devices for interpretation and documentation

- ◆ UV source (220 - 310 nm)
- ◆ camera (e.g. Polaroid system) with films (Polaroid type 667) or video system with thermal paper (e.g. Typ KP65HM-CE)
- ◆ PC, evaluation software HISTO MATCH (BAG Health Care) or SCORE (full version)

## 2.3. Storage and stability

The HISTO TYPE kits are delivered at ambient temperature. Upon receipt please store the kits at  $\leq -20^{\circ}\text{C}$  in the dark. The HISTO TYPE plates/strips can also be stored at 2...8°C, but frequent changes of the storage temperature should be avoided. The 10x PCR buffer must be stored at  $\leq -20^{\circ}\text{C}$ .

The Happy Taq will be shipped with dry ice or cool packs. Store the Happy Taq at  $< -20^{\circ}\text{C}$ .

Store in temperature monitored devices.

The expiry date is indicated on the label of each reagent and is also valid for opened reagents. The expiry date indicated on the outer label refers to the reagent with the shortest stability contained in the kit.

### 3. Data of performance

The composition of the primer mixture guarantees a reliable identification of the HLA-types (based on the latest sequence data) indicated in the hit table. Updates will be done regularly.

The accuracy and reproducibility of the specificity of each primer mix were verified for each lot with pre-typed reference samples. Not recognized alleles are indicated in the evaluation documents.

Performance evaluation studies with pre-typed DNA samples have been performed for all HISTO TYPE SSP Kits. The results were compared to results with other HLA-SSP-Kits, sequencing or serological HLA test methods. The typing results showed 100% concordance with the pretyping results.

The evaluation and quality control of the mixes are done with DNA samples, which were extracted by EXTRA GENE I (salting out method) or Qiagen QIAamp DNA Blood Mini and Maxi kits (column based method). When another DNA extraction Kit is used, the suitability of the extracted DNA for the application with the HISTO TYPE kits must be validated by the user.

The HISTO TYPE kits are validated with the Happy Taq (REF 70976). When another Taq Polymerase is used, the enzyme must be validated with the HISTO TYPE kits by the user.

A reliable typing can be guaranteed if 25 - 50 ng DNA per reaction mix are used.

### 4. Test procedure

#### 4.1 Safety conditions and special remarks

The PCR is a particularly sensitive method and should be performed by well trained personnel, experienced in molecular genetic techniques and histocompatibility testing. Transplantation guidelines as well as EFI standards should be followed in order to minimize the risk of false typings, particularly in the case of discrepancies between serological and molecular genetic methods.

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).
- ◆ Use separate working areas for pre-amplification (DNA isolation and preparation of the reactions) and post-amplification (gel electrophoresis, documentation). Preferably, use two separate rooms.
- ◆ Use devices and other materials only at the respective places and do not exchange them.

## 4.2 DNA isolation

The sample material for the isolation of genomic DNA should be sent in appropriate blood collection systems. The presence of heparin potentially inhibits PCR; therefore blood collection systems with heparin are not suitable [6]. EDTA or Citrate blood is recommended for typing.

### Validate DNA extraction methods:

- EXTRA GENE I (BAG)
- QIAGEN QIAamp DNA Blood Mini and Maxi Kit

Laboratory established standard method for DNA isolation should be validated by the user.

A DNA concentration of 25-50 ng/μl is required to perform the test.

DNA should have the following purity indexes:

- $OD_{260}/OD_{280} = >1.5$  and  $<2.0$  (indicator for contamination with RNA/proteins)
- $OD_{260}/OD_{230} = >1.8$  (indicator for contamination with salt, carbohydrate or organic solvents)

## 4.3 Amplification

All prealiquoted and dried reaction mixtures already contain allele and control-specific primers and nucleotides. These are supplied dried down in the reaction vial. Amplification parameters are optimized to a final volume of 10 μl.

1. Thaw the 10 x PCR buffer shortly before use.
2. Remove the required number of HISTO TYPE plates / strips from the kit.
3. Pipet the Master-Mix consisting of 10x PCR buffer, DNA solution, Taq-Polymerase and Aqua dest. and mix well. The different HISTO TYPE SSP Kits do all work with the same Master-Mix and can therefore be combined. The composition of the Master-Mix depending on the number of reaction mixes is given in Table 1 (see below).

In case of **HISTO TYPE B27** it's recommended to set up a **Taq-buffer-H<sub>2</sub>O solution**:

<b>0.08 μl</b>	Taq polymerase (5 U/μl)	x no. determinations + 1
<b>1.0 μl</b>	10x PCR buffer	x no. determinations + 1
<b>7.0 μl</b>	H <sub>2</sub> O	x no. determinations + 1

Please mix the solution thoroughly and add **8,0 μl** of it to each reaction vessel.

Afterwards add **2.0 μl DNA** solution (12.5-25 ng/μl) into the respective reaction vial.

If a **contamination control** should be performed, produce the Master-Mix without the DNA solution first and pipet 10 μl of this mix in the reaction mix for the contamination control (coloured blue). Afterwards add the DNA solution to the remaining Master-Mix and mix well.

**Table 1: Composition of the Master-Mix depending on the number of reaction mixes**

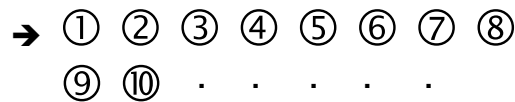
no. of mixes	Aqua dest.	10x PCR buffer	DNA-solution (25-50 ng/µl)	Happy-Taq (5 U/µl)	whole volume	
1	8	1	1	0.08	10	µl
4	63	8	8	0.6	80	µl
8	79	10	10	0.8	100	µl
24	222	28	28	2.2	280	µl
30	269	34	34	2.7	340	µl
32	285	36	36	2.9	360	µl
48	412	52	52	4.2	520	µl
54	459	58	58	4.6	580	µl
56	475	60	60	4.8	600	µl
72	618	78	78	6.2	780	µl
80	681	86	86	6.9	860	µl
96	808	102	102	8.2	1020	µl

⇒ The quantity of DNA must be 25 – 50 ng per mix. According to the DNA-concentration, the amount of DNA and water have to be adjusted (e.g. for 24 mixes: 14 µl DNA solution (100 ng/µl) and 236 µl Aqua dest.).

⇒ If another Taq Polymerase shall be used, the enzyme must be validated with the HISTO TYPE kits by the user.

4. After vortexing add **10 µl** of this mixture immediately to the pre-dropped and dried reaction mixtures. Change the tip after each pipetting step. Tightly close the tubes with the respective caps or foil. Ensure that you do not touch the inner side of the caps and the upper edges of the tubes with the fingers to

Marking



avoid contamination. If cyclers with tightly closable lid are used, it is also possible to use reusable PCR mats. Slightly shake the plate/strip to dissolve the pellet at the bottom of the plate/strip. All PCR solution should settle on the bottom. If necessary the plate/strip should be briefly spun down.

**Note for HISTO TYPE B\*57:01/B\*51**

**If only B\*57:01 or only B\*51 should be detected, add the Master-Mix only to the corresponding reaction mixes (specificity of the reaction mixes see specificity table on Information CD).**

4. Place the reaction tubes firmly into the thermal cycler and tighten the lid well. Start the PCR programme. Overlaying of the reaction mixtures with mineral oil is **not** required if a heated and adjusted lid is used!

**Amplification parameters**

Programme-Step	Temp.	Time	No. of Cycles
First Denaturation	96°C	5 Min	1 Cycle
Denaturation	96°C	20 Sec	5 Cycles
Annealing+Extension	68°C	1 Min	
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

**Validated Cycler types:**

PTC 100 / 200 / C1000  
(MJ Research/ BioRad),  
GeneAmp PCR-System 9600  
/ 9700 (use heating rate of  
9600 please), Veriti (ABI),  
Mastercycler epGradient S  
(use "simulate Mastercycler  
gradient" function please)  
(Eppendorf),  
Tprofessional (Biometra)

**Please don't use an aluminium heating block (e.g. GeneAmp PCR-System 9600 / 9700).**

**When using thermal cyclers with a very fast heating and cooling rate, it is recommended to use a reduced ramp rate (~ 2.5°C/sec).**

**Since cyclers of different manufacturers perform differently and even individual machines of one type may be calibrated differently, it may be necessary to optimize the amplification parameters. If other models than the validated cyclers mentioned above are used they have to be validated by the user.**

To optimize your machine use the following guide:

If there are **false positive** reactions (unspecific bands, additional types), increase the annealing temperature in 1°C steps.

If there are **false negative** reactions (bands missing), decrease the annealing temperature in 1°C steps and/or increase the annealing times in 5 second steps and/or increase the denaturation times in 5 second steps.

**It is recommended to use exclusively cyclers that are calibrated regularly. For this the CYCLER CHECK kit is well suited (REF 7104, 71044).**

**The quality control tests were done on a PTC-200 resp. C1000 (MJ Research / BioRad), 9700 (ABI), Mastercycler epGradient S (Eppendorf) and Tprofessional (Biometra).**



#### 4.4 Gel electrophoresis

Separation of the amplification products is done by electrophoresis via a (horizontal) agarose gel. As electrophoresis buffer, 0.5 x TBE (45 mM of tris, 45 mM of boric acid, 0.5 mM of EDTA) buffer is recommended. The gel concentration should be 2.0 - 2.5% of agarose. Allow the gel to polymerize at least 30 minutes before sample loading. After amplification has been finished, take the samples out of the thermal cycler and load the complete reaction mixtures carefully in each slot of the gel. In addition, apply 10 µl of the DNA length standard for size comparison.

Electrophoretic separation is done at 10 - 12 V/cm (with 20 cm distance between the electrodes approx. 200 - 240 V), for 20 - 40 minutes. After the run has been completed, the complete gel is stained in an ethidium-bromide (EtBr) solution (approx. 0.5 µg/ml of EtBr in H<sub>2</sub>O or TBE buffer) for 30 - 40 minutes. As alternative, EtBr (0.5 µg/ml) can also be added to the electrophoresis buffer or to the agarose gel. If required, excess of EtBr can be removed by soaking the gel in H<sub>2</sub>O or 0.5 x TBE buffer for 20 - 30 minutes.

#### 4.5 Documentation and interpretation

For documentation, visualize the PCR amplification using an UV transilluminator (220 - 310 nm) and photograph it with a suitable camera, film and filters (e.g. Polaroid, film type 667 or video system, thermal paper KP65HM-CE). Choose exposure time and aperture such that the bands are drawn sharp and stand out against the dark background. For interpretation see the specificity table and hit table (see Information CD); only bands that have the correct size compared to the DNA length standard should be considered positive.

**With HISTO TYPE B27, the specific bands have a length of 420 bp and/or 85 bp.**

In case of the other HISTO TYPE products the correct sizes are given in the evaluation documents. In all lanes without allele-specific amplification, the **1070 bp** internal control (**at HISTO TYPE Celiac Disease 1070bp / 429bp**) should be clearly visible. In most cases with an allele specific amplification the internal control is weaker or disappears completely!

If neither a specific band nor the internal control band appears, the result with the relevant mix can not be used for evaluation. Possible reasons for not evaluable results see troubleshooting (6.).

No band should be visible in the **contamination control**. If there is a contamination with genomic DNA there will be a band at 282 bp. Additional bands may occur at 78 bp, 104 bp, 176 bp and around 580 bp. If there is a contamination with amplicates bands will occur at 78 bp and/or 104 bp and/or 176 bp and/or 282 bp and/or 580 bp.

For the evaluation the HISTO MATCH (free of charge by BAG Diagnostics) or SCORE (full version) software must be used (except for HISTO TYPE B27 low and HISTO TYPE Narcolepsy).

Batch files for the evaluation with HISTO MATCH and SCORE see Information CD. The files are also available from the download server (<http://service.bag-diagnostics.com>) or via our customer service (phone: +49 (0) 6404-925-125).

## 5. Warnings and Precautions

Ethidiumbromide is a powerful mutagen. Wear gloves when handling gels or solutions containing EtBr! Note the instructions for use and warnings and precautions of the manufacturer!

The transilluminator radiates very short-wave UV light which may cause burns of the skin and the retina. Use a UV face safety mask!

All biological material used for extraction of DNA, e.g. blood or human tissue, 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.

A declaration on Material Safety Data Sheets (MSDS) is available to download at [www.bag-diagnostics.com](http://www.bag-diagnostics.com).

## 6. Troubleshooting








Problem	Possible Reason	Solution
no amplification, length standard visible	DNA contaminated with PCR-inhibitors	repeat DNA isolation, try different methods
	DNA concentration too high/too low	alter DNA concentration, repeat DNA isolation
	enzyme is missing or concentration too low	repeat typing, alter enzyme concentration
	DNA from heparinized blood	repeat typing with EDTA or citrate blood
	wrong amplification parameters	optimize the amplification parameters (see 4.3) ☆
repeated failure in single lanes (no amplification- control)	leak in reaction tubes; water loss and change in concentration during PCR	close tubes tight with caps
unspecific amplification, additional bands, (additional bands of the wrong size must be neglected)	contamination with amplification products	repeat typing, ensure exact working
	DNA contaminated with salts	repeat DNA isolation, try different methods
	DNA concentration too high	use less DNA
	enzyme concentration too high	use less enzyme
	wrong amplification parameters	optimize the amplification parameters (see 4.3) ☆
evaluation shows more than 2 specificities	carry-over contamination (amplification products!) new allele	check typing mixtures (no DNA added) ensure exact working
no or only very weak bands visible, length standard invisible	staining too weak	repeat staining
gel background shines too bright	staining was too long, staining solution concentration too high	soak gel in H <sub>2</sub> O or TBE lower staining solution concentration
blurred band	electrophoresis buffer too hot or used up, wrong electrophoresis buffer, polymerisation of the gel not well	lower the voltage use 0.5x TBE buffer use completely polymerised gel

☆ When using the equipment and materials listed, optimization of the amplification parameters should be looked upon as a last resort. In most cases, it is possible to evaluate the test by eliminating the additional bands caused by size discrepancies.

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## 8. Explanation of symbols used on the labels

	Storage temperature / Temperature limitation
	Storage temperature / Lower limit of temperature
	Use by
	Consult instructions for use
	Sufficient for n tests
	Caution
	Manufacturer
<b>CONT</b>	Content, contains
<b>CONTROL   CC</b>	Contamination Control
<b>eIFU</b>	Electronic instructions for use
<b>HLA TYPING</b>	Intended use: HLA typing
<b>HISTO TYPE INFORMATION CD</b>	CD (contains instructions for use, files for evaluation, quality control certificate)
<b>IVD</b>	For in vitro diagnostic use
<b>LOT</b>	Batch code
<b>OR</b>	Or
<b>PCRBUF   10x</b>	PCR buffer, 10x concentrated
<b>PCRCAP</b>	PCR caps
<b>PCRFOIL</b>	PCR foil
<b>PCRPLATE</b>	PCR plates
<b>PCRSTRIP</b>	PCR strips
<b>REACTIONMIX</b>	Reaction mixes
<b>REF</b>	Catalogue number
<b>RTU</b>	Ready to use
<b>TAQ POLYMERASE</b>	Taq-Polymerase
<b>WORKSHEET</b>	Worksheet

Instructions for use in other languages see:

<http://www.bag-diagnostics.com> or contact us directly at [info@bag-diagnostics.com](mailto:info@bag-diagnostics.com)

or phone: +49 (0)6404-925-125