UK

HLA SSP

Package size

HLA-A SSP	REF 826 201	CE 0197	24 PCR reactions
HLA-B SSP	REF 826 206	CE 0197	48 PCR reactions
HLA-C SSP	REF 826 212	CE	18 PCR reactions
DRB SSP	REF 826 215	CE 0197	24 PCR reactions
DQB SSP	REF 826 220	CE	8 PCR reactions
ABDR SSPtray	REF 826 230	CE 0197	96 PCR reactions
ABC SSPtray	REF 826 240	CE 0197	93 PCR reactions

Short Manual

Before first using an HLA SSP kit please check the detailed manual available from http://www.bio-rad.com!

BLOCK PCR-Block NC Negative control CK PCR-Cocktail

Intended Use
HLA Sequence Specific Primers (SSP) kits are intended for the determination of HLA Class I alleles.

Reagents
Contents of the HLA SSP Kits
The contents of the HLA SSP Kits are sufficient for 24 tests.

- 24 PCR-blocks BLOCK respectively, each consisting of PCR tubes in blocks that contain the dried primer/nucleotid mixtures. As an aid to identification, a black mark has been placed onto position 1 (=H1).
- PCR cocktail CK (ready for use)
  The cocktail contains PCR-buffer, glycerol and cresol red.
- PCR cover strips or PCR cover seals.
- Worksheets, reaction pattern chart, primer position sheet
- The negative control NC is packed as additional PCR tubes (colourless) , if it is not included in the PCR block BLOCK.

Warning and Precautions
For In Vitro Diagnostic Use IVD

Caution: The test must be performed by well-trained and authorised aboratory technicians.
Caution: All reagents should be handled in accordance with good laboratory practice using appropriate precautions. In addition, handle all patient samples as potentialially infectious. Do not pipette by mouth.
Caution: Do not use reagents past the expiration date printed on the label.
Caution: Do not use reagents with any evidence of turbidity or microbial contamination.
Caution: Pipettes used for **Post**-PCR manipulations should **not** be used for **Pre**-PCR manipulations.
Caution: Biohazard Warning: The ethidium bromide used for staining of DNA is a potential carcinogen. Always wear protective gloves when handling stained gels. Waste management by burning.
Caution: Biohazard Warning: All blood products should be treated as potentially infectious.
Caution: All used PCR blocks should be treated as potentially infectious and should be destroyed according to the valid national guidlines.
Caution: Wear UV-blocking eye protection, and do not view UV light source directly when viewing or photographing gels.

See Material Safety Data Sheets for detailed information.

Storage and Shelf Life
The SSP reagents (BLOCK, CK, NC) must be stored at 2...8 °C. The expiry date is printed on the packaging of the kit components. PCR blocks are sealed in pouches.

Sample
DNA Quantity
The DNA sample to be used should be resuspended in sterile distilled water at a concentration of approx. 100 ± 50 ng/µl. DNA **should not be re-suspended** in solutions containing chelating agents, **such as EDTA,** above 0.5 mM in concentration.

DNA Quality
For good PCR-SSP results, DNA is required with an A_{260}/A_{280} ratio of ≥1.6.

➲ The purity and concentration of the DNA is of decisive importance for optimal test results.

Performing the HLA SSP typing test
For HLA typing of one DNA sample PCR reactions with a reaction volume of 10 µl in each PCR tube are performed. The black mark serves as an aid for correct orientation of the PCR block BLOCK (position H1).

Prepare for **each typing test** a **master mix** containing the following components:

PCR cocktail CK
Taq DNA polymerase (5 U/µl)
dH_2O

Mix well and pipette 10 µl of this mixture to the negative control NC. After that add **sample DNA** (approx. 100 ± 50 ng/µl) and mix well.

See the following table for different master mix configurations:

	Master mix for configuration with				
Number of PCR reactions	8	18	24	48	96
PCR cocktail CK	44 µl	100 µl	120 µl	228 µl	440 µl
Taq DNA polymerase	0.7 µl	1.5 µl	1.8 µl	3.5 µl	7 µl
dH_2O	55 µl	125 µl	150 µl	288 µl	550 µl
DNA (approx. 100 ± 50 ng/µl)	11 µl	25 µl	30 µl	57 µl	110 µl

From this master mix, pipette 10 µl to each of the dried primer mixes. This is best done using a multipette. Care should be taken that the pipette tips do not make contact with the primer in order to avoid carry-over of the primer. For this reason, pipette the master mix to the walls of the well.

Seal properly the PCR strips. Transfer the tray into the thermal cycler and start the PCR with the program HLA SSP.

Program HLA SSP:

Initial Denaturing:	**94°C**	**2 min.**	
Denaturing:	**94°C**	**10 sec.**	⎤
Annealing & Extension:	**65°C**	**60 sec.**	⎦ **10 cycles**
Denaturing:	**94°C**	**10 sec.**	⎤
Annealing:	**61°C**	**50 sec.**	⎥
Extension:	**72°C**	**30 sec.**	⎦ **20 cycles**

Gel Electrophoresis
The PCR products are identified using agarose gel electrophoresis followed by detection of the DNA bands in UV light.

A 2 % solution of agarose is prepared by boiling 5 g of agarose in 250 ml of 1x TBE until the solution becomes completely dissolved. By stirring the solution cool it down to < 60°C and add 4µl of ethidium bromide. Subsequently pour the agarose solution – devoid of bubbles - into a prepared and sealed gel tray. Place the combs (10µl pockets) and keep it at room temperature for at least 10 minutes.
After the agarose has solidified the gel is inserted into the gel chamber. The combs are removed and the gel is covered with 1 x TBE. The gel pockets should be completely covered by the buffer. Pipette the entire PCR mixtures (10 µl) into the gel pockets.
To be able to check the size of the PCR products, the use of an appropriate molecular weight standard (50-1000bp marker) is recommended for the electrophoresis.
The electrophoresis takes place in 15 to 25 minutes at 8V/cm (distance between electrodes). The migration distance of the cresol red should amount to 1-1.5cm.

After the completion of the electrophoresis the gel is placed on an UV transilluminator and photographed for documentation and interpretation.
Attention: Wear UV-blocking eye protection, and do not view UV light source directly when viewing or photographing gels.

Evaluation
The HLA primer mixture contains control primers which amplify a 1069 bp fragment of human growth hormone (HGH). The concentration of these primers is lower than the allele-specific primer pairs and their purpose is to provide an internal control of successful PCR amplification. This amplification generally always occurs, i.e. both in the presence and in the absence of an allele- or group-specific PCR fragment. The control band can therefore generally be seen in all PCR reactions. From time to time, the control band can appear weak or is completely missing in the presence of an allele-specific HLA PCR product. This is not a limitation to the method, as the specific band provides a check on the success of the PCR run.
The composition of the primers permits positive identification of the HLA characteristics. The interpretation is based on whether a specific band is present on the gel or not. The size of the amplified DNA fragments does not need to be taken into consideration when evaluating the test, nevertheless it might be helpful for the test interpretation.
For evaluation, the pattern of the specific bands is transferred to the result sheet supplied and the typing result read off with the aid of the reaction pattern or with the aid of the Bio-Rad HLA-SSP Typing Software (REF 847 075) and the lot update available on the Bio-Rad homepage.

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189738001/08 – 04/2016