## HLA SSP Typing Kits

| Product | REF | Package | |
|---|---|---|---|
| HLA-A | 800 111 | 24 Tests | CE 0197 |
| HLA-B | 800 112 | 24 Tests | CE 0197 |
| HLA-DR | 800 113 | 24 Tests | CE 0197 |
| HLA-C | 800 114 | 24 Tests | CE |
| HLA-DQ | 800 115 | 24 Tests | CE |
| HLA- DRDQ | 800 116 | 24 Tests | CE 0197 CE |
| HLA-ABC | 800 117 | 20 Tests | CE 0197 CE |
| HLA-ABDR | 800 110 | 20 Tests | CE 0197 |
| HLA-ABDRDQ | 800 118 | 20 Tests | CE 0197 CE |

Short Instructions for Use
If you are using the HLA SSP Typing Kits for the first time please read the detailed instructions for use carefully!

**Intended Use:**
The HLA SSP Typing Kits are a "low resolution" test system for the identification of HLA alleles from DNA using the PCR method.

**Contents of the Set**

- HLA PCR Plates containing the dried Primer/dNTP-Mixes. In one corner a black dot is applied as an orientation point.
- 2.5x PCR Mix (ready for use).
- The PCR Mix contains PCR buffer, cresol red, glycerol and detergents.
- PCR cover sheets (adhesive).
- Worksheet, Reaction pattern, primer position sheet
- Instructions for Use, Short Instructions for Use

**General Safety Instructions**

- For in-vitro diagnostic (IVD) use only
- The test has to be performed by well-trained and authorized qualified personnel.
- All reagents and probes should be handled as potentially infectious and the appropriate precautions should be taken.
- Use different pipettes for the Post- and the Pre-PCR area.
- Caution: ethidium bromide for dyeing the agarose gels is potential carcinogenic. Allow no contact to the skin; always wear special nitrile protective gloves while working.
- Caution: Wear UV blocking glasses for eye protection. Avoid direct UV light when viewing or taking pictures of agarose gels.
- Do not use reagents past the expiration date printed on the labels.
- Do not use reagents under suspicion of turbidity or microbial contamination.
- Used PCR plates are considered as potentially infectious and like the agarose gels should be destroyed according to the prevailing national guidelines.

For Material Safety Data Sheets for the HLA SSP Typing Kits make application at the R.O.S.E. Europe GmbH.

Storage and Shelf Life
The HLA SSP PCR plates and the PCR-Mix have to be stored at 2 °C - 8 °C. The expiration day is printed on all labels. Opened packages should be used within 4 weeks.

**DNA**
The test requires DNA with a ratio ($A_{260}/A_{280}$) of >1.6 and the DNA should be used preferentially at a concentration of 75 ng (± 25 ng) per reaction.

**Performing the SSP Typing Test**
Preparing the master mix following the table below:

| Amount of PCR-Reactions | **24** | **32** | **48** | **96** |
|---|---|---|---|---|
| PCR Mix | 110µl | 148µl | 220µl | 440µl |
| Taq DNA Polymerase [5 U/µl] | 1,8µl | 2,4µl | 3,5µl | 7µl |
| DNA [50 ng/reaction] | 27,5µl | 37µl | 55µl | 110µl |
| Water (bidest) | 135,7µl | 182,6µl | 271,5µl | 543µl |

- Pipette together PCR-Mix, bidest. Water and Taq DNA Polymerase. Mix the DNA thoroughly and add the appropriate amount to the solution. Mix well.

- Pipette 10 µl of the master mix in every well of the PCR plate containing the dried primer/dNTP-Mixes. For this purpose we suggest the use of a dispenser. Carefully pipette the master mix on the wall at the top of the well. This way ensures that the specific primers are not carried over from one well to another. The black dot serves as a control point.

- Spin down the solution and seal properly with the PCR adhesive foil.

- Put the plate into the thermal cycler and start the program below.

| | Cycle | Temp. | Time |
|---|---|---|---|
| | Denaturation | 94 °C | 2 min |
| 10 cycles | Denaturation | 94 °C | 10 sec |
| | Annealing and Extension | 65 °C | 1 min |
| 20 cycles | Denaturation | 94 °C | 10 sec |
| | Annealing | 61 °C | 50 sec |
| | Extension | 72 °C | 30 sec |
| | Elongation | 72°C | 2 min |
| | Final Hold | 4 °C | ∞ |

**Gel Electrophoresis**
The PCR products are identified using agarose gel electrophoresis and detected through ethidium bromide intercalation under UV light.

- Preparing a 2 % agarose gel:
For one gel use 2 g agarose in 100 g 1X TBE buffer. Cook the solution till the agarose is completely dissolved. Cool the solution to approximately 50 °C and fill up with distilled water to 100 g. Add 1 µl ethidium bromide solution [10 mg/ml]. Caution: **ethidium bromide** is **potentially carcinogenic**. No contact to the skin, always carry special protective gloves!
Pour the agarose solution free of bubbles into a prepared gel tray, insert the combs for 96 wells and let it solidify at room temperature for at least 15 min.

- Performing the electrophoresis:
After the gel is solidified, transfer it into the gel chamber, remove the combs and cover it completely with 1x TBE buffer. Transfer the complete volume of the PCR from the plate (10 µl) into the gel pockets (take notice of the marking on the plate). To check the size of the DNA fragments use an appropriate molecular weight standard (100-1000 bp) (optional). Connect the chamber to a power supply and run the electrophoresis with the settings 8 V/cm (electrode gap) ~ 20 min. The migration of the cresol red in the gel should be ~1-1.5 cm.

- Interpretation:
Following the electrophoresis the products are made visible by UV light and photographed for interpretation with a gel documentation system.
Caution: **Wear UV blocking glasses for eye protection**. Never look directly into the UV light source, while viewing or taking pictures of the gels.

**Evaluation**
In addition to the Sequence Specific Primers (SSP) the primer mixes contain at a lower concentration control primers of the ubiquitous human globin gene. They act as an internal control for the PCR. For the HLA-A, HLA-B and HLA-C PCR the length of this fragment is 1070 bp and for the HLA-DRB and HLA-DQ it is 429 bp. The control band should be present in every successful PCR. If the SSP product is positive, the control band can be weaker or even entirely missing, because the reaction with the positive primer in the well is prefered.

For the interpretation of the results it is important if a band is present in the gel or not. The pattern of the positive bands identifies the HLA characteristics. The size of the fragments (by using a molecular weight standard) can help for the interpretation of the results, but it is not essential for the evaluation of the test. The interpretation should be performed with the delivered worksheet and reaction pattern. The usage of the right version has to be observed, do not use a version older than six months. For the current version of the worksheet and the reaction pattern make application at the R.O.S.E. Europe GmbH.

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