SSP UniTray®

Instructions for Use

For Both Low and High Resolution Kits

SSP UniTray®

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For In Vitro Diagnostic Use

The Invitrogen™ SSP UniTray® is a PCR-based method designed to provide low to high resolution of the various HLA Class I and II types. Formulations of allele or group specific primer sets are used to amplify genomic DNA using a 96 well thermal tray. Setup includes mixing a reaction buffer with a human genomic DNA sample and Taq DNA Polymerase*, dispensing the mixture to the UniTray®, sealing and then thermal cycling. After cycling is complete, the PCR products are loaded onto a 2% agarose gel for electrophoresis. After electrophoresis, the ethidium bromide stained gel is photographed and interpreted using a worksheet for the specific amplification patterns. The test can be completed in 2.5 hours post DNA isolation. (Times vary depending on make and model of the thermal cycler used).

The SSP UniTray® method is based on sequence specific primer amplification methods (SSP) previously published. [1-3] The primer sets amplify the alleles described by the international nomenclature committee of WHO.[1] For details, see the Worksheet, Primer Mix Specificity Table and Ambiguity List provided with each kit. The method has been extensively tested with reference DNA from the International Workshops; UCLA Reference DNA Panel samples and other well-characterized, serotyped samples.

*Taq DNA Polymerase, Ampli-Taq and the Gene-Amp PCR process are subject of patents and patent applications of Hoffmann-LaRoche, USA.

In this manual:

1 Kit Components:

	Description	Quantity	Storage
1.1	96 well polycarbonate PCR trays containing 5 µl/well of optimized primer solution overlaid with paraffin oil	2 to 10 trays (kit dependent)	-20° C in a **NON-FROST FREE FREEZER**
1.2	75-580 µl aliquots of optimized PCR Buffer containing dNTPs and Gel Loading Buffer	10-40 vials (kit dependent)	-20° C in a **NON-FROST FREE FREEZER**
1.3	Plastic sealers for sealing PCR trays	3 – 11 (kit dependent)	Room temperature
1.4	Gel Documentation Form	10-40 (kit dependent)	---------
1.5	Worksheets	11-41 (kit dependent)	---------
1.6	Primer Mix Specificity Table	1	---------
1.7	Certificate of Analysis/Tray Configuration	1	---------
1.8	Taq Polymerase, 5 units/µl (optional)	Kit dependent	-20° C in a **NON-FROST FREE FREEZER**

2 Material, Reagents, and Equipment not Supplied:

2.1 Taq DNA Polymerase, 5 units/µl:

2.1.1 The following enzymes are validated for use with the SSP UniTray® products: **Invitrogen**™ Recombinant Taq DNA Polymerase; **Roche Molecular Systems**, Taq DNA Polymerase; **Perkin Elmer**, Ampli-Taq DNA Polymerase; **Fisher**, Taq DNA Polymerase; **Advanced Biotechnologies Ltd**., Taq DNA Polymerase. Use of other DNA polymerase enzymes must be validated by the user.

2.1.2 For the purchase of kits with Taq Polymerase and License fees, please refer to the Invitrogen™ Product Catalog, or contact your Sales Representative. See the last page of this manual for an important notice regarding kits sold with Taq Polymerase.

2.2 Sterile, molecular grade water

2.3 Pipettors and tips: 1-10 µl
10-200 µl
100-1000 µl

2.4 Dispensing electronic pipettors: 100-250 µl capacity, capable of dispensing 8 µl aliquots

2.5 8 channel pipettor: 5-25 µl adjustable volume

2.6 96 well thermal cycler with heated lid:

e.g., **MJ Research**, PTC-100 Peltier, PTC-200 DNA Engine, PTC-225 DNA Engine Tetrad; **Perkin Elmer**, Gene Amp 9600; **Perkin Elmer**, Gene Amp 9700; and the **LabLine**, Thermal Block II Model 212.

Note: Kits have been tested with the above thermal cyclers. Use of different equipment will require user validation of thermal cycling parameters.

2.7 Invitrogen™ Heat Equalizing Block, Product Code # 900001D

2.8 TBE electrophoresis buffer (at 0.5X concentration)

2.9 DNA Molecular Weight markers to cover range of 50 – 2000 bp

2.9.1 Invitrogen™ PCR Markers, Product Code # 74601250 (recommended)

2.10 Invitrogen™ DNA Grade Agarose, Product Code # 75000500 (recommended)

2.11 Ethidium bromide (10 mg/ml)

Caution: Ethidium bromide is a mutagen. Handle with appropriate personal protective equipment.

2.12 Electrophoresis Power supply

2.13 Recommended Electrophoresis System:

2.13.1 Electro-Fast® Electrophoresis System: 96 sample lanes plus separate marker loading lanes.
This product can be purchased through Invitrogen™ Product code #920001.

2.13.2 Owl Centipede™ Extra Wide Minigel System, Model #: D3-14 with 4 Microtiter combs, 25 teeth, 1.5 mm thick.
This product can be purchased through Invitrogen™ Product Code #800001

2.14 Recommended Gel Documentation System:

2.14.1 UV transilluminator

2.14.2 Polaroid camera with hood and filter for gel documentation

2.14.3 Polaroid film type 667

3 Sample Requirements:

3.1 DNA Sample in TE buffer or sterile water

1.05-10 µg total DNA for one full typing (kit dependent), equivalent to 14-80 µl (kit dependent) at 75-125 ng/µl of DNA (see **Table 5.0 at Sample Setup Section 5**).

3.2 DNA isolated from blood samples should be collected in EDTA or ACD anticoagulated tubes. **DO NOT USE HEPARINIZED SAMPLES.** Heparin may inhibit DNA amplification.

3.3 Good quality DNA is critical to achieve an optimal result with the SSP UniTray® System. Good quality DNA means that:

3.3.1 The $OD_{260/280}$ is between 1.7 and 1.9 on diluted sample measured by UV spectrophotometry

3.3.2 When checked on agarose gel electrophoresis, the major portion of DNA runs slower than the 9.4 kb band of Hind III digested Lambda DNA marker

Note: DNA isolation may be performed by any qualified protocol that produces high purity and good quality DNA. The Invitrogen™ DNA Isolation Kit (Product Code #761001) and QIAamp® System are validated for this use.

4 Thermal Cycler: SSP UniTray® Amplification Profile:

4.1 It is important to obtain rapid ramp times (~1° per second) and precise temperature control for optimal results.

4.1.1 If using the Perking Elmer PE9700 thermal cycler, set ramping speed to the Perkin Elmer PE9600 setting.

4.1.2 If using the MJ Research thermal cyclers, select the temperature control to **calculated**, not **block.**

4.2 The following thermal cycler profile is optimized and validated for use with the UniTray® SSP product line (see Section 2.6):

Step 1	1 minute at 96º C
Step 2 5 cycles of	96º C 25 seconds 70º C 50 seconds 72º C 45 seconds
Step 3 21 cycles of	96º C 25 seconds 65º C 50 seconds 72º C 45 seconds
Step 4 4 cycles of	96º C 25 seconds 55º C 60 seconds 72º C 120 seconds
Hold	4º C specify time

Total reaction volume (reaction + paraffin oil overlay) in each well = **23 µl**

Note: The UniTray® is designed to be placed directly in the thermal cycler unit. Do not use a tray holder or tray retainer.

5 Sample Setup:

Note: PCR buffer is aliquotted in single test volumes. Use one vial per test.

Use the following reference table (Table 5.0) to aid in PCR setup:

TABLE 5.0

Number of Reactions Per Typing	Vol. of Aliquotted PCR buffer (µl)	Taq DNA Polymerase (µl)	Water (µl)	Vol. of DNA (75-125 ng/µl) Added to Mix (µl)	Final Vol. of DNA and Water (µl)
2-8 wells	75.0 (1 vial)	1.2	30.0	14.0	44.0
9-12 wells	85.0 (1 vial)	1.4	34.0	15.0	49.0
13-16 wells	120.0 (1 vial)	1.9	48.0	20.0	68.0
17-24 wells	150.0 (1 vial)	2.4	60.0	25.0	85.0
25-32 wells	200.0 (1 vial)	3.2	80.0	34.0	114.0
33-40 wells	250.0 (1 vial)	4.0	100.0	42.0	142.0
41-44 wells	275.0 (1 vial)	4.4	110.0	46.0	156.0
45-48 wells	300.0 (1 vial)	4.8	120.0	50.0	170.0
96 wells	580.0 (1 vial)	9.3	268.0	80.0	348.0

5.1 Water Control Tube.

5.1.1 Many laboratory accreditation standards require that a setup contamination control reaction (Water Control Tube) be performed with each typing.

5.1.2 Add 50 µl of molecular reagent grade water to a clean 0.5 ml (or larger) polypropylene tube.

5.1.3 Place the OPENED tube containing the 50 µl of water to one side of the test setup area and proceed with test setup.

5.2 Thaw frozen PCR buffer (one vial for each test).

5.3 Remove one tray from freezer.

5.3.1 Record Tray Identification Number indicated on pouch label on the Gel Documentation Form.

5.3.2 Carefully remove the SSP UniTray® from its pouch.

5.3.3 If only a single typing is to be run, use scissors to cut the UniTray® between columns of adjacent tests (See product table Certificate of Analysis for specific UniTray® primer mix layout). Return remaining tests to pouch. Store at original conditions. Use within one month.

Note: When cutting a tray, cut from right to left, avoiding cutting off the letters (A-H) in the leftmost column which aid in determining proper UniTray® orientation.

5.3.4 Do not allow paraffin oil to thaw before cutting or removing seal as this may cause mix dispersal.

5.3.5 Place the tray inside a sample holder, such as a clear microtiter plate or a 0.2 ml tube rack (8 x 12).

5.3.6 Carefully remove adhesive seal from SSP UniTray®.

5.4 Remove Taq DNA Polymerase from freezer and keep chilled during setup (e.g., on ice).

5.5 Add water and Taq DNA Polymerase to the PCR buffer (from section 5.2) and mix thoroughly. (See Table 5.0 for appropriate volumes of water and Taq).

5.6 ***Note: Thaw tray completely before adding PCR buffer mixture.*** Remove 7 µl from this mixture (Section 5.5) and add to the contamination control well of the individual typing. The contamination control well is the last well of each primer mix set. See Kit Tray Configuration for exact well position(s).

5.7 Add 1µl of water from the water control tube (see Section 5.1) to the contamination control well referenced in Section 5.6 above.

5.8 Add the volume of completely dissolved DNA sample (75-125 ng/µl) to the remaining buffer mixture, as indicated by Table 5.0, and mix thoroughly.

5.9 Using an electronic dispensing pipettor, dispense 8 µl into each of the remaining wells. Be careful to dispense the drops onto the side walls of the wells, near each well's top, allowing the dispensed drop to slide under the paraffin oil. Do not allow the pipette tip to come in contact with the well contents.

Note: Confirm that each well contains sample by noting the color of the solution in each well. A settled sample will be indicated by a purple solution color. If a drop is hung up on the side of a well, GENTLY tap the tray in the holder against the bench top to ensure proper mixing of the DNA sample and primer. Sufficient volume is supplied to allow for pipetting losses.

5.10 Remove backing from an adhesive plastic seal and place over the top of the tray.

5.10.1 Gently press the seal onto the tray, making sure that the tray is completely sealed.

5.10.2 Trim the edges of the plastic seal, if necessary. It is normal for the plastic seal above the wells to appear indented upon completion of the thermal cycling run. This does not affect amplification in any way.

5.11 Set the tray in thermal cycler and place the Heat Equalizing Block on top of the sealed tray. Close the lid and tighten. Begin thermal cycling.

Note: It is very important that the sealed tray be seated firmly in the thermal cycler so that all wells are in contact with the block. It is necessary to place the Heat Equalizing Block on top of the sealed plate to ensure firm contact and heat transfer. This is required even when using a heated lid. Do not use the sample holder supplied by Perkin Elmer with the SSP UniTray®. See Troubleshooting (Section 9.4): Overall poor or absent amplification.

Note: Sections 5.9 through 5.11 must be performed efficiently to minimize time between sample addition and initiation of thermal cycling. Prolonged incubation at room temperature (greater than 5 minutes) may cause mispriming and nonspecific PCR reactions.

5.12 After thermal cycling, remove tray and proceed to gel electrophoresis. If not performing electrophoresis immediately, store tray at 4° C for up to one week.

6 Gel Electrophoresis:

General Directions: Use a high quality agarose, capable of resolving 50-2000 base pair fragments of DNA. Invitrogen™ DNA Grade Agarose (Product Code #75000500) works well at 2%. Prepare the gel in 0.5X TBE buffer. After cooling to 60° C add 2 µl of 10 mg/ml ethidium bromide for each 100 ml agarose solution and mix well. Pour into casting tray and allow to cool for at least 30 minutes. Use 0.5X TBE buffer in gel chamber as a running buffer. Gels can be run at 10 volts per centimeter gel length.

The following directions are for the Owl Centipede™, Extra Wide Minigel System only. Other gel systems will require different amounts of agarose and electrophoresis conditions. Consult your specific equipment protocol for assistance, or contact technical support personnel at Invitrogen™. **If using the Electro-Fast® Electrophoresis System, follow the instructions for electrophoresis included with these units.**

6.1 Pour a 160 ml 2% agarose gel following the guidelines above. Use four 25 well microtiter format combs and place one at the top and the others at equal distances below.

6.2 Fill electrophoresis chamber with 0.5X TBE buffer.

6.3 Carefully remove seal from UniTray®.

6.3.1 Holding the tray firmly inside a holder, carefully fold back the seal from one edge.

6.3.2 **Caution:** Sudden movement of the tray can disperse amplified product and oil, contaminating the laboratory and may require repetition of the test.

Note: It is recommended that the tray be cooled at 4° C for five minutes to let the paraffin oil solidify before the seal is removed. This will help prevent accidental dispersal of amplified product during seal removal.

6.4 Load 5 µl PCR marker to the appropriate lane(s) of the gel (**See Gel Documentation Form**).

If using the Electro-Fast®, carefully load 2 µl of the PCR marker into the designated wells.

6.5 Using an 8 channel pipettor, carefully transfer 8 µl of PCR products/gel loading buffer from the tray, begin with wells A-1 through H-1, to the gel lanes (See Gel Loading Template 6.5).

If using the Electro-Fast®, carefully load 6 µl of the PCR product into the wells.

Note: Be certain to keep the tips at the bottom of the well while slowly drawing up sample into the pipette tips. With a paper towel, blot off any oil remaining in the tips. Gently load the samples into the gel.

Gel Loading Template 6.5

	1	2	3	4	5	6	7	8	9	10	11	12
A	lane 1											
B	lane 2											
C												
D												
E												
F												
G												
H												

See Certificate of Analysis for Kit Specific Tray Configuration

Note: The word "well" refers to the tray location assignment, while the word "lane" refers to a well's corresponding gel lane.

6.6 Electrophoretically separate the DNA at 150 volts for 18-23 minutes using the Owl Centipede™ unit, or until the orange dye front in the Invitrogen™ PCR Marker approaches the next row of wells.

Note: The purple sample dye will be at approximately 300 bp after running the gel for 18-23 minutes.

6.7 Turn off power, disconnect electrodes and remove gel. Photograph gel over UV-transilluminator.

Note: Use of the Invitrogen™ Fluorescent Numbering Panel may aid in positive lane identification. Order the Centipede™ system Numbering Panel by specifying Product Code # 810011. Order the Electro-Fast® system Numbering Panel by specifying Product Code # 920011.

Note: To aid in troubleshooting and technical support it is helpful for Invitrogen Corporation to obtain an original gel photo. For this purpose, the user may want to take additional photos.

7 Interpretation:

7.1 Affix the gel photo to the Gel Documentation Form.

7.1.1 On the Gel Documentation Form, “M” refers to the marker lane.

7.2 Examine gel photo carefully and determine the positive lanes.

7.2.1 Each lane of the gel, containing a loaded sample, should show a control band except the lane which contains the contamination control well. See the Gel Documentation Form for details on the internal control sizes.

7.2.2 The control band may or may not amplify efficiently when there is specific product present due to substrate competition. The control primers are present in lower concentration in order to favor the allele specific reaction.

7.2.3 Weak bands above the internal control (except the 200 base pair internal control) may appear in all lanes. These are not of particular concern. They are an indication of robust amplification.

7.2.4 Absent control bands with no specific amplification are indicative of failed reactions.

7.2.4.1 If alleles can be determined in the presence of a failed PCR reaction, and that failed reaction does not change the allele assignment, the test does not need to be repeated.

7.2.4.2 If, however, there is an apparent homozygous result, or the missed reaction could change an allele assignment, the typing must be repeated.

7.2.5 If weak bands of incorrect product size are present, disregard them if the overall strength and clarity of the amplification is good.

7.2.6 Unused primers will form a diffuse band below 50 base pairs.

7.2.7 Primer dimer usually appears above the primer band, but below the area where specific product is found; this appears as a fuzzy band below 80 base pairs.

7.2.8 Several lanes have two or more possible sizes of PCR products. These wells have multiplexed primer pairs which give rise to different, amplicons depending upon the allele present. Refer to the Primer Mix Specificity Table for further information for allele assignment.

7.2.9 See Primer Mix Specificity Table and ambiguity list provided with each kit for details on resolution.

7.2.10 False negative reactions can be caused by inefficient amplification, poor quality of DNA, uneven placement of the plate in the block, temperature variations across the wells of the thermal cycler itself, or inadequate thermal cycler calibration.

7.2.10.1 False negative reactions rarely occur when the control band is present

7.2.10.2 It is possible that the false negatives are due to a new or yet uncharacterized allele.

7.2.11 The contamination control well contains primer pairs that amplify DNA produced by either specific PCR amplifications or genomic DNA.

7.2.11.1 If the negative buffer/Taq mixture was added as directed, any band in this lane is evidence of contamination and the results of the test are invalid.

7.2.11.2 A primer dimer band of <80 base pairs may be present. This **does not** invalidate the test. Primer dimers are known to occur occasionally.

7.3 Confirm the approximate product size using the Gel Documentation Form or Worksheet.

7.4 Mark the positive lanes on the worksheet.

7.5 Using a highlighting pen, highlight each positive lane (column) with a vertical line running through the chart columns from top to bottom.

7.6 Align a ruler across the first row of the worksheet and scan from the left to right. Examine each row from top to bottom, making the first allele assignment only where all the black boxes for that allele choice fall within the highlighted lanes.

7.7 Assign the second allele choice if needed by continuing to scan the remaining alleles as above. Be sure to complete an entire review of the worksheet to determine all possible allele assignments. **You must account for all positive lanes at least once.** However, lanes can be used to assign more than one allele.

7.8 Be certain that all the lanes required for a particular allele are positive before making a specific allele assignment. Refer to Section 9 for reaction patterns that do not give a typing result.

7.9 For High Resolution SSP UniTray®, assignments should not be made for allele groups exhibiting positive reactions other than the groups for which the test was designed, i.e., B*15 assignments should not be made from a B*35 High Resolution SSP UniTray®.

8 Limitations and Precautions:

8.1 Before implementing the SSP UniTray® method in your laboratory, perform quality assurance and quality control for amplification based methods using known **molecularly** typed samples. Such samples can be obtained from the International Workshop Reference Cell Panel and the UCLA DNA Reference panel. ***Be sure to consult the Primer Mix Specificity Table and Ambiguity List for details on coverage.***

8.2 Every attempt has been made to validate all primer mixes used in this kit with molecularly typed DNA samples or well-characterized serologically typed samples. Due to the lack of access to molecularly typed reference material, some wells may not have been validated using positive control DNA. Refer to the Certificate of Analysis for detailed information.

8.3 HLA typing using the Invitrogen™ SSP UniTray® must be performed in the presence of a qualified Director, Technical Supervisor and/or general Supervisor following accepted laboratory accreditation standards. We must emphasize that these products are for professional use only.

9 Troubleshooting:

General Problems

9.1 Use of excess sample DNA (200 ng/µl or more per reaction) may favor nonspecific PCR products. An intense smear of high molecular weight DNA present on gel photos of amplified products may indicate that excess DNA was used. A general weak amplification might indicate that less than the required amount of sample DNA was used in the reaction (<75 ng/µl per reaction) which may cause false negative reactions.

9.2 RNA contamination may cause overestimation of DNA concentration when measured by spectrophotometry. A way to verify the reading is to run a small aliquot of sample DNA (about 200 ng) in a 0.7% agarose gel and compare it with a DNA marker of known concentration.

9.3 Degraded DNA may not amplify reliably with UniTray®. Verify DNA integrity as above. Obtain another sample and repeat the DNA extraction. For technical details, please contact Invitrogen Corporation.

9.4 **Problem:** Overall poor or absent amplification indicated by weak control bands, absent or negative allele specific bands.

(Possible Causes)

9.4.1 Inadequate contact between thermal cycler block and tray – DO NOT use the tray holder or tray retainer provided by Perkin Elmer with the UniTray®.

9.4.2 Heparinized samples – use EDTA or ACD as anticoagulants.

9.4.3 Poor quality DNA – when using the Invitrogen™ DNA Isolation Kit, clean up DNA by repeating protease digestion, or re-purify the DNA sample. As a last resort, extract a fresh sample.

9.4.4 Low DNA concentrations –use more DNA and adjust the water volume accordingly to buffer mixture or concentrate DNA.

9.4.5 Inhibitors present – make sure that DNA is of good quality (see section 3.3) before setting up PCR.

9.4.6 Degraded DNA sample – is apparent by presence of a smear in the gel lanes. Isolate DNA from a fresh sample.

9.4.7 Improperly calibrated thermal cycler – recalibrate thermal cycler.

9.4.8 Lack of Taq DNA Polymerase activity – verify activity of Taq with a known reference DNA sample.

9.4.9 Annealing temperature not optimal – decrease the annealing temperature in step 2 of the amplification profile from 70º C to 69º C and in step 3 of the amplification profile from 65º C to 64º C (Section 4.2).

9.5 **Problem**: Random failures: more than 1 failed lane

(Possible Causes)

9.5.1 DNA is not evenly re-suspended in diluent – pipet DNA up and down several times to aid mixing; alternatively, heat DNA on a heat block 10 minutes at 70º C to dissolve.

9.5.2 DNA not mixed adequately with PCR buffer –mix thoroughly before adding to tray.

9.5.3 Uneven volume of buffer/Taq/DNA solution added – exercise caution when dispensing samples. Make sure all of the reaction mixture is contained under the paraffin oil.

9.5.4 Inadequate contact between thermal cycler block and tray – DO NOT use the tray holder or tray retainer provided by Perkin Elmer with UniTray®.

9.6 **Problem:** False positives

(Possible Causes)

9.6.1 Excess DNA or Taq Polymerase - measure DNA with UV spectrophotometry.

9.6.2 Extensive delay between PCR setup and start of thermal cycling – no more than a 5 minute delay should be allowed before thermal cycling.

9.6.3 Incorrect order in gel loading – check alignment of mixes and gel lanes.

9.6.4 Interpretation of primer dimer as specific bands – check correct band size.

9.7 **Problem**: False negatives

(Possible Causes)

9.7.1 Improperly calibrated thermal cycler – recalibrate thermal cycler.

9.7.2 If recalibration does not correct the problem, re-test the sample with a previously typed reference sample with the same allelic specificity. If confirmed as negative, call Invitrogen Corporation for technical support.

9.7.3 Failure of buffer/Taq/DNA drop to pass through paraffin oil. Briefly centrifuge tray before thermal cycling or tap gently on bench top.

9.7.4 Incorrect order in gel loading – check alignment of mixes and gel lanes.

9.7.5 Annealing temperature not optimal – decrease the annealing temperature instep 2 of the amplification profile from 70º C to 69º C and in step 3 of the amplification profile from 65º C to 64º C.

9.8 **Problem:** Overall fuzzy bands, smeared lanes

(Possible Causes)

9.8.1 Gel is too thin due to excess evaporation while heating – compensate for lost volume by adding water.

9.8.2 Agarose not completely dissolved – boil for an additional 30 seconds after melting.

9.8.3 Overheating gel, too high voltage – lower voltage.

9.8.4 TBE concentration too high – concentration should be 0.5X TBE.

9.8.5 Heavy streaking in random wells can be caused by uneven suspensions of DNA – using an 8 channel pipettor, mix the PCR product up and down two times before loading.

9.8.6 Rapid release of amplified product during gel loading can cause product to float out of well – use slow, steady pipetting when loading gel.

9.9 **Problem**: Gel picture too dark

(Possible Causes)

9.9.1 Forgot to add ethidium bromide, or added the wrong amount – use 2 µl ethidium bromide (10 mg/ml) for each 100 ml agarose solution.

9.9.2 Gel tray not UV transparent – remove gel from tray before viewing.

9.9.3 Incorrect camera setting – increase exposure time or aperture setting.

9.10 **Problem**: Gel picture too bright

(Possible Causes)

9.10.1 Excess amount of ethidium bromide – use 2 µl ethidium bromide for each 100ml agarose solution.

9.10.2 Incorrect camera setting – decrease exposure time or aperture setting.

9.11 **Problem**: Occasional faint lanes

(Possible Causes)

9.11.1 Product floated out of well – pipette tips need to be properly aligned with gel wells.

Disclaimer:

NOTICE TO PURCHASER: SSP UniTray® Kits without Taq Polymerase – This product is optimized for use in the Polymerase Chain Reaction ("PCR") Process which is covered by patents by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd. ("Roche"). No license under these patents to use the PCR Process conveyed expressly or by implication to the purchaser by the purchase of this product. Further information on purchasing licenses to practice the PCR Process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, CA 94501.

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References:

1. Current HLA alleles can be found at: http://www.anthonynolan.org.uk/HIG/index.html

2. Bunce M., O'Neil C., Barnardo M., Morris P., Welsh K. Phototyping: Comprehensive DNA typing for HLA-A, B, C, DRB 3, DRB 4, DRB 5 and DQB 1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP) Tissue Antigens V 46 November 1995.

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Self-Declared Products (CE marked)	
451404	DQB1 SSP UniTray Kit - for HLA tissue typing
451414	DQB1 SSP UniTray Kit with Taq Polymerase - for HLA tissue typing
451606D	DPB1 SSP UniTray Kit - for HLA tissue typing
451616D	DPB1 SSP UniTray Kit with Taq Polymerase - for HLA tissue typing
451703	DQA1 SSP UniTray Kit - for HLA tissue typing
457173	DQA1 SSP UniTray Kit with Taq Polymerase - for HLA tissue typing
4719010	Cw High Res SSP UniTray Kit - for HLA tissue typing
4719110	Cw High Res SSP UniTray Kit with Taq Polymerase - for HLA tissue typing
7830010	C LOCUS SSP UniTray Kit - for HLA tissue typing
7830110	C LOCUS SSP UniTray Kit with Taq Polymerase - for HLA tissue typing

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