# UniTray® DNA Wipe Test

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# Product Overview

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

## Product description

### Kit usage

The UniTray® DNA Wipe Test is a PCR-based method designed to monitor DNA contamination in the laboratory that may interfere with accurate reporting of test samples. The UniTray® DNA Wipe Test has the capacity to detect human genomic DNA and the majority of previously amplified DNA generated from sequences containing exon 2 and/or exon 3 of HLA A, B, C and exon 2 of the DRB, DQA, DQB, DPA, DPB genes.

To use the UniTray® DNA Wipe Test, you will:

1. Collect specimen samples from areas throughout the laboratory.
2. Make a master mix (containing reaction buffer and *Taq* DNA Polymerase).
3. Dispense the master mix and samples to trays containing pre-aliquotted Contamination Control Primer mix
4. Thermal cycle.
5. Load the PCR reactions onto a 2% agarose gel for electrophoresis.
6. Photograph the ethidium bromide stained-gel.
7. Analyze the photograph and gel to determine presence of contaminants.

The test can be completed in 2.5 hours post specimen sample collection. (Times vary depending on make and model of the thermal cycler used.)

# Sensitivity

The UniTray® DNA Wipe Test has been established to be at least as sensitive as UniTray SSP mixes in detecting amplification products from UniTray® and AllSet+™ Gold mixes and SeCore® amplification mixes. The primer sets and nucleotide positions used for contamination detection from the Class I and Class II UniTray®, AllSet+™ Gold or SeCore® Kits are listed in the following tables.

## Class I: A, B, C

| Sense Primer 3'end Sequence | Sense Primer 3'end Position | Antisense Primer 3'end Sequence | Antisense Primer 3'end Position |
|---|---|---|---|
| GAC | 162 | AGA | 299 |
| GAC | 162 | GGC | 392 |
| GAC | 162 | AGA | 228 |
| GAC | 162 | AGA | 541 |
| YGG | 208 | AGA | 299 |
| YGG | 208 | GGC | 392 |
| YGG | 208 | AGA | 228 |
| YGG | 208 | AGA | 541 |
| GGA | 341 | GGC | 392 |
| GGA | 341 | AGA | 541 |
| ACA | 442 | AGA | 541 |

## Class II: DRB/DQA/DQB/DPA/DPB

- The PCR product sizes range from 50–655 bp.
- The 3-nucleotide sequence is the target sequence of the sense and the antisense primers, which together determine the specificity of the primer mix. Primer locations are referenced to the IMGT/HLA alignments

| Sense Primer 3'end Sequence | Sense Primer 3'end Position | Antisense Primer 3'end Sequence | Antisense Primer 3'end Position |
|---|---|---|---|
| GAA | 145 | TGG | 227 |
| GAA | 177 | TGG | 227 |
| CGA | 209 | ACG | 232 |
| CGA | 209 | GGA | 263 |
| TGA | 158 | GAR | 189 |
| TGA | 158 | GAR | 189 |
| CCG | 197 | GGA | 278 |
| CCG | 197 | GCG | 241 |
| CGA | 224 | GGA | 278 |
| CGA | 224 | GCG | 241 |
| GTT | 133 | ACG | 238 |
| GTT | 133 | GGA | 269 |
| GCG | 178 | ACG | 238 |
| GCG | 178 | GGA | 269 |
| CGA | 215 | ACG | 238 |
| CGA | 215 | GGA | 269 |

# UniTray® DNA Wipe Test contents and storage

| Item | Description | Quantity | Storage |
| --- | --- | --- | --- |
| 96-well polycarbonate PCR trays | Each well contains 5 µL of contamination control primer mix overlaid with paraffin oil. | 2 plates | −30°C to −10°C in a non-frost-free freezer |
| Positive control DNA | 20 µL at a concentration of 50 ng/µL. | 8 each | −30°C to −10°C in a non-frost-free freezer |
| Aliquots of optimized PCR buffer | 120 µL aliquots of optimized PCR Buffer containing dNTPs and Gel Loading Buffer. | 8 vials | −30°C to −10°C in a non-frost-free freezer |
| PCR sealing sheets | Self-adhesive plastic sheets for sealing test plates. | 3 sheets | — |
| Plastic Applicators | — | 100 applicators | — |
| Wipe test record sheets | — | 10 sheets | — |
| CD ROM | • Product manual<br>• Worksheet<br>• Certificate of Analysis | 1 disk | — |

# Materials and equipment required but not included

<table>
<tr><th>Item</th><th>Source</th></tr>
<tr><td>Taq DNA Polymerase, 5 units/µL</td><td>The following enzymes are validated for use with the UniTray® DNA Wipe Test:<br>• Life Technologies Recombinant Taq DNA Polymerase<br>• Roche Molecular Systems Taq DNA Polymerase<br>The validated Taq DNA Polymerases used with the UniTray® DNA Wipe Test have the following characteristics:<br>• Non Hot Start<br>• 5′ to 3′ polymerase activity<br>• 5′ to 3′ exonuclease activity<br>• Does not have 3′ to 5′ exonuclease activity<br>Note: Other DNA polymerase enzymes must be validated by the user.</td></tr>
<tr><td>96-well thermal cycler with heated lid</td><td>96-well x 0.2-mL thermal cycler with heated lid:<br>• Temperature range ≥ 4°C to 99.9°C<br>• Thermal Accuracy of +/– 0.75°C<br>• Ramping speed of ≥ 1°C per second<br>Note: This kit has been validated with thermal cyclers that meet the aforementioned specifications. Using different equipment will require user validation of thermal cycling parameters.</td></tr>
<tr><td>Recommended Electrophoresis System</td><td><table>
<tr><th>Item</th><th>Life Technologies Cat. no.</th></tr>
<tr><td>E-Gel® 96 2% Agarose 8-Pak</td><td>A10570</td></tr>
<tr><td>E-Gel® 48 2% Agarose 8-Pak</td><td>A10571</td></tr>
<tr><td>Electro-Fast™ Electrophoresis System: 96 sample lanes plus separate marker loading lanes</td><td>920001</td></tr>
<tr><td>Owl Centipede™ Extra Wide Minigel System, Model #: D3-14 with 4 Microtiter combs, 25 teeth, 1.5-mm thick</td><td>800001D</td></tr>
</table></td></tr>
<tr><td>Recommended Gel Documentation System</td><td>UV transilluminator</td></tr>
</table>

# General purpose supplies required but not included

All of the items in this table can be purchased from any major laboratory supplier.

| Item | Description |
| --- | --- |
| Sterile, molecular grade water | — |
| 1.5 mL sterile polypropylene tubes | — |
| Pipettes and tips | • 1–10 µL<br>• 10–200 µL<br>• 100–1000 µL |
| Dispensing electronic pipettes | 5–25 µL capacity, adjustable volume |
| Heat Equalizing Block | Life Technologies Cat. no. 900001D |
| DNA Molecular Weight markers to cover range of 50–2000 bp | Life Technologies PCR Markers, Cat. no. 74601250 (recommended) |
| DNA Grade Agarose | Life Technologies Cat. no. 75000500 (recommended) |
| TBE electrophoresis buffer | 0.5X concentration |
| Ethidium bromide<br>Caution: Ethidium bromide is a mutagen. Handle with appropriate personal protective equipment. | 10 mg/mL |
| Electrophoresis Power supply | — |

# Methods

## Before starting

### Collect samples

Test several commonly-used areas for contamination, including the DNA prep room, the PCR setup area, and the post-amplification area.

1. In a PCR-clean area, label 1.5-mL tubes for each of the sample areas. We recommend that you include work benches where DNA extraction and PCR setup is performed, pipette handles, centrifuges, freezer handles, door knobs, microtube racks, etc.
2. Using sterile, aerosol-free (filtered) pipette tips, aliquot 300 µL ultra pure, sterile, water into each tube. Wet a sterile, plastic applicator swab in each tube.
3. Wipe a 10-cm square sample area with the moistened applicator, and place the applicator back into the original tube. Snap off the plastic end of the applicator and cap the tube. Vortex briefly.

   Note: Set up samples from the post-PCR area in an area other than the pre-PCR area. We recommend you set up the PCR for the UniTray® Wipe Test in an isolated location to prevent contamination of the pre-PCR area.
4. Incubate the samples in a 55° C water bath for 1 hour.
5. Centrifuge the tubes for 1 minute at the highest speed in a microcentrifuge (~14,000 × *g*).
6. Using sterile forceps carefully remove and discard applicators from the tubes.

## Sample setup

1. Remove the tray from the freezer and cut the desired number of tests from the tray (see the following section, **Recommended Test set** up).
2. Carefully remove the adhesive seal from the tray and allow the mixes to thaw completely.

## Recommended Test set up

| | | Test 1 | | | Test 2 | | | Test 3 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Controls | A | ● | ● | ○ | ● | ● | ○ | ● | ● | ○ | ● | ● | ○ |
| Sample #1 | B | ● | ● | ○ | ● | ● | ○ | ● | ● | ○ | ● | ● | ○ |
| Sample #2 | C | ● | ● | ○ | ● | ● | ○ | ● | ● | ○ | ● | ● | ○ |
| Sample #3 | D | ● | ● | ○ | ● | ● | ○ | ● | ● | ○ | ● | ● | ○ |
| … | E | ● | ● | ○ | ● | ● | ○ | ● | ● | ○ | ● | ● | ○ |
| … | F | ● | ● | ○ | ● | ● | ○ | ● | ● | ○ | ● | ● | ○ |
| … | G | ● | ● | ○ | ● | ● | ○ | ● | ● | ○ | ● | ● | ○ |
| … | H | ● | ● | ○ | ● | ● | ○ | ● | ● | ○ | ● | ● | ○ |

Inhibition (column 2)
Sample (column 1)

●- Indicates wells containing primer mix
○- Indicates wells without primer mix
A1,A4,A7,A10= positive control wells
A2,A5,A8,A11= negative control wells

### Notes

- Each tray contains 4 tests of aliquotted contamination control primer mix.
- Each test contains a well for a positive and negative control, and 7 sample and inhibition control wells. An example of our recommended set-up would be A1-positive control; A2-negative control; B1-sample #1; B2-sample #1 inhibition control, etc.
- All wells contain the same primer mix. If you do more or less than 7 wipes, cut the tray to accommodate the number of sample areas that you need.

## Pre-Amplification set up

### PCR mix

- Dependent on the number of PCR reactions per test.
- Prepare a master mix for amplification. Use the following volumes for each set-up, including sample areas, inhibition controls, positive and negative controls, plus 1–2 additional reaction volumes to compensate for pipetting losses.

| Number of wells per test | PCR Buffer (µL) | Water (µL) | *Taq* (µL) |
|---|---|---|---|
| 41–48 | 300 | 120 | 4.8 |
| 33–40 | 250 | 100 | 4 |
| 25–32 | 200 | 80 | 3.2 |
| 17–24 | 150 | 60 | 2.4 |
| 13–16 | 120 | 48 | 1.9 |
| 9–12 | 85 | 34 | 1.4 |
| 2–8 | 75 | 30 | 1.2 |

# Amplification

## Prepare and load the tray

1. Add 1μL of water to the negative control well.
2. Vortex and add 1μL Positive Control DNA (50 ng/μL) to the positive control well, making sure to pipet the control to the bottom of the well.
3. Add 1μL Positive Control DNA to each inhibition control well.
4. Add 1μL of test area sample to both corresponding test sample wells (sample reaction and inhibition control wells).

   Note: Inhibition control wells will have a final volume of 24 μL.
5. Remove the *Taq* DNA Polymerase from the freezer and keep chilled during setup (e.g., on ice).
6. Add water and *Taq* DNA Polymerase to the thawed PCR buffer and mix thoroughly. (See the table on page 12 for appropriate volumes of water and *Taq*).
7. Dispense 7 μL into each well. 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.

   The total reaction volume (reaction + paraffin oil overlay) in each well = **23 μL**
8. Remove the backing from an adhesive plastic seal and place it over the top of the tray.
   a. Gently press the seal onto the tray, making sure that the tray is completely sealed.
   b. 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.

      Note: Perform steps 1 through 8 efficiently to minimize the 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.

## PCR amplification set up (perform in the post-PCR area)

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

   Note: The UniTray® tray is designed to be placed directly in the thermal cycler unit. Do not use a tray holder or tray retainer. Seat the sealed tray firmly in the thermal cycler so that all wells are in contact with the block. 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 UniTray® Wipe Test.

2. Program the thermal cycler using the following parameters:

| Step | Temperature (°C) | Time (seconds) | Action |
|---|---|---|---|
| A denaturation step | 96 | 60 | Denature |
| 5 cycles | 96<br>70<br>72 | 25<br>50<br>45 | Denature<br>Anneal<br>Extend |
| 21 cycles | 96<br>65<br>72 | 25<br>50<br>45 | Denature<br>Anneal<br>Extend |
| 4 cycles | 96<br>55<br>72 | 25<br>60<br>120 | Denature<br>Anneal<br>Extend |
| Hold | 4 | User-specified time | — |

   Note: Obtain rapid ramp times (~1°C per second) and precise temperature control for optimal results. For specific thermal cycler information, refer to the manufacturer's handbook.

3. Start the program.

4. When the program is complete, remove the samples from the thermal cycler.

   Note: After thermal cycling, remove the tray and proceed to gel electrophoresis. If you are not performing electrophoresis immediately, store the tray at 4ºC for up to 1 week.

# Gel electrophoresis

## General directions

Use the following procedure to detect PCR amplification using agarose gel electrophoresis.

## Procedure

### Prepare a 2% agarose gel

1. Add 2 g of agarose (Life Technologies Cat. no. 75000500) to 100 mL 0.5x TBE buffer to prepare a 2% (w/v) agarose gel.
2. Dissolve the agarose by boiling in a microwave oven until thoroughly dissolved.
3. Cool to 60°C.
4. Add ethidium bromide to a final concentration of 0.5 µg/mL gel.
5. Cast a 3–4 mm thick gel.
6. Allow the gel to set for at least 30 minutes.

### Run the gel

1. Transfer the agarose gel to a submarine gel electrophoresis unit.

   Note: Cover the gel with 0.5X TBE buffer to a depth of approximately 1–2 mm above the gel surface.
2. Carefully remove the seal from the UniTray® tray. Holding the tray firmly inside a holder, carefully fold back the seal from one edge.

   Caution: Sudden movement of the tray can disperse amplified product and oil, contaminating the laboratory, and possibly necessitating repetition of the test.

   Note: Cool the tray at 4º C for 5 minutes to let the paraffin oil solidify before removing the seal. This will help prevent accidental dispersal of amplified product during seal removal.
3. Load 8µL of the PCR products into the wells of the 2% agarose gel.

   Note: Do not add gel loading buffer to the samples.

   - If you are using the Electro-Fast® Gel System, carefully load 6 µL of the PCR product into the wells.
   - 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.
4. Load 5 µL of PCR marker to the appropriate lane(s) of the gel. (See the Gel Documentation Form.)

   Note: If you are using the Electro-Fast® Gel System, carefully load 2 µL of the PCR marker into the designated wells.

5. Run the gels at 10 volts per centimeter gel length. For the Owl Centipede™ unit, electrophoretically separate the DNA at 150 volts for 18–23 minutes or until the dye front in the 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. Turn off the power, disconnect electrodes and remove the gel.

# Interpretation

| Observation | Explanation |
|---|---|
| Amplified product ranging in size from 50–655 base pairs | Contamination is present. A positive band of any size in this range should be considered positive. |
| No observed bands | No detectable contamination is present.<br>Note: The inhibition control reaction must be positive for the sample reaction to be valid. |
| 200, 800, or 1200 bp bands | There is contamination from internal control bands. The UniTray® DNA Wipe Test detects amplification products from the UniTray®, AllSet+™ Gold or SeCore® Kits using a method at least as sensitive as these routine test methods. |
| The inhibition control band is weaker than the positive control | An inhibitor may be present in that sample. Dilute the sample from 1:10 to 1:50 in sterile double distilled water and repeat the test following the same protocol. |

## Notes

- Conditions for the UniTray® DNA Wipe Test may not be optimal for every laboratory. You may need to adjust the amount of the positive control DNA used in the test to obtain the best positive control results.
- If you detect contamination:
  1. Cleanse the area with a fresh 10% bleach solution.
  2. Allow the area to dry.
  3. Wipe the area clean with distilled water.
  4. Retest the area.

# Appendix

## General Safety Warning

⚠ **WARNING! GENERAL SAFETY.** Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the “Documentation and Support” section in this document.
- All testing should be performed in accordance with local, regional and national acceptable laboratory accreditation standards and/or regulations.

## Chemical safety

⚠ **WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

## Biological hazard safety

WARNING! – BIOHAZARD**.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/ 29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/ WHO_CDS_CSR_LYO_2004_11/en/**

# Documentation and Support

## Obtain SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**

For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

## Obtain support

For the latest services and support information for all locations, go to:

**www.lifetechnologies.com/support**

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**HLATechSupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

## Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on the CD enclosed with the kit.

## Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

## Symbols

| Symbol | Description |
|---|---|
| | Manufacturer |
| Σ | Number of Tests |
| | Consult Instructions for Use |
| | Temperature Limitation (range) |
| | Lower Temperature Limitation |
| | Upper Temperature Limitation |
| | Use By |
| REF | Catalog Number |
| LOT | Batch Code |
| Read SDS | Read Safety Data Sheet |
| | Keep Away from Sunlight |
| | Warning: Product may contain biohazardous material |
| SN | Serial Number |
| | Date of Manufacture |
| EC REP | Authorized Representative in the European Community |
| | Warning: Attention, see instructions for use |
| | Caution, Risk of Electric Shock |

# References


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2. Bunce M., O'Neil C., Barnardo M., Morris P., Welsh K. Phototyping: Comprehensive DNA typing for HLA-A, B, C, DRß 3, DRß 4, DRß 5 and DQß 1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP) Tissue Antigens V 46 November 1995.
3. Bodmer, J.G., Marsh, S.G.E., Ekkehard, D. A. et al. Nomenclature for factors of the HLA system, 1996. Human Immunology V 53: 98-129, March 1997.
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