AutoGenomics

INFINITI® CYP450 2D6I Assay

Directional Package Insert (DPI)

For *In Vitro* Diagnostic Use

CE

FOR EXPORT ONLY

Manufactured by AutoGenomics, Inc., 2980 Scott Street, Vista, CA USA 92081

Authorized EU Agent: Medical Device Safety Service GmbH (MDSS)
Schiffgraben 41, 30175 Hannover, Germany

AutoGenomics

INTENDED USE

The INFINITI CYP450 2D6I Assay is an *in vitro* diagnostic test for the identification of a patient's CYP450 2D6 genotype from genomic deoxyribonucleic acid (DNA) obtained from whole blood samples. The INFINITI CYP450 2D6I Assay is a qualitative assay for use in clinical laboratories upon prescription by the attending physician.

The INFINITI CYP450 2D6I Assay is indicated for use as an aid to clinicians in determining therapeutic strategy for therapeutics that are metabolized by the CYP450 2D6 gene product.

The information provided from this test may supplement decision making and should only be used in conjunction with routine monitoring by a physician. Because of the variability in the knowledge of clinical utility with specific drugs that are metabolized by CYP450 2D6, clinicians should use professional judgment in the interpretation of results from this test. Results from this type of assay should not be used in predicting a patient's response to drugs for which the drug metabolizing enzyme activity of the allele, or the drug metabolic pathway, has not been clearly established.

BACKGROUND INFORMATION

Cytochrome P450 (CYP) is a major group of drug-metabolizing enzymes (DME) that consists of more than 50 isoforms. The majority of CYP activity takes place in the liver. CYP2D6 shows the largest phenotypical variability amongst the CYPs, largely due to genetic genotype accounts for normal, reduced and non-existent CYP2D6 function in subjects.

CYP2D6 is one of the most important DME genes as it metabolizes 25% to 30% of all prescribed drugs. Common drug categories metabolized by CYP2D6 include (but are not limited to): beta blockers, anti-arrhythmics, morphine derivatives, and antidepressants.

The CYP2D6 function in any particular subject may be described as one of the following:

- poor metabolizer - these subjects have little or no CYP2D6 function.
- intermediate metabolizers - these subjects metabolize drugs at a rate somewhere between the poor and extensive metabolizers.
- extensive metabolizer - these subjects have normal CYP2D6 function.
- ultra-rapid metabolizer - these subjects have multiple copies of the *CYP2D6* gene expressed, and therefore greater-than-normal CYP2D6 function.

The genetic basis for extensive and poor metabolizer variability is the *CYP2D6* allele, located on chromosome 22. Subjects who possess certain allelic variants will show normal, decreased or no CYP2D6 function depending on the allele. Ethnicity is a factor in the occurrence of CYP2D6 variability. Table 1 lists the CYP2D6 alleles and corresponding enzyme activity. Table 2 provides the distribution of some CYP2D6 Alleles by race/ethnicity.

Table 1

***CYP2D6* allele and enzyme activity**	
Allele	CYP2D6 activity
*CYP2D6*1*	normal
*CYP2D6*2*	increased
*CYP2D6*3*	none
*CYP2D6*4*	none
*CYP2D6*5*	none
*CYP2D6*6*	none
*CYP2D6*7*	none
*CYP2D6*8*	none
*CYP2D6*9*	decreased
*CYP2D6*10*	decreased
*CYP2D6*11*	none
*CYP2D6*15*	none
*CYP2D6*17*	decreased
*CYP2D6*19*	none
*CYP2D6*20*	decreased
*CYP2D6*29*	decreased
*CYP2D6*35*	normal
*CYP2D6*36*	decreased
*CYP2D6*40*	none

Table 2

Variant	Phenotype (2D6 Function)	Allele Frequency (Percentage)		
		Caucasian	Black/African	Asian
CYP2D6*3	Poor Metabolizer	2	0	0
CYP2D6*4	Poor Metabolizer	12 - 21	2	1
CYP2D6*4XN	Poor Metabolizer	2 - 7	4	6
CYP2D6*10	Intermediate Metabolizer	1 - 2	6	51
CYP2D6*17	Intermediate Metabolizer	0	34	0

CYP450 2D6 genetic testing can be useful for identifying individuals who may have an adverse drug reaction or a poor response to a medication.

TEST PRINCIPLE/ASSAY OVERVIEW

The INFINITI CYP450 2D6I Assay is an *in vitro* diagnostic test for the multiplex detection of the genotypes of CYP2D6 in deoxyribonucleic acid (DNA) obtained from human blood samples.

The INFINITI CYP450 2D6I Assay is designed to detect and identify the following allelic variants:

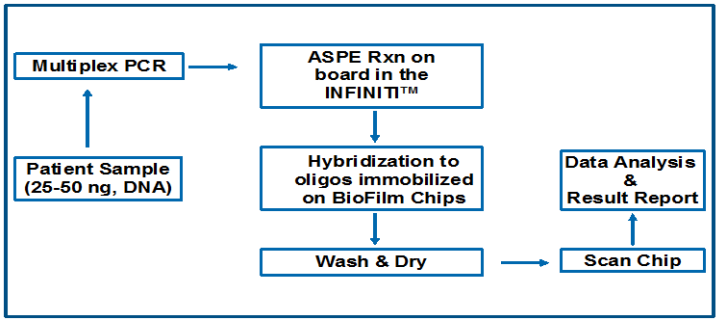
Gene	Polymorphism	
CYP450 2D6	*2 (2850C>T)	*9 (2615_2617delAAG)
	*3 (2549delA)	*10 (100C>T)
	*4 (1846G>A)	*12 (124G>A)
	*5 (CYP2D6 deleted)	*17 (1023C>T)
	*6 (1707delT)	*29 (1659G>A)
	*7 (2935A>C)	*41 (2988G>A)
	*8 (1758G>T)	*XN (multiple CYP2D6)
	*14 (1758G>A)	*2A(-1584C>G)

The INFINITI CYP450 2D6I Assay is based on the following processes:

a) DNA extraction from human blood sample.
b) PCR amplification of purified DNA.
c) Fluorescent label incorporation using analyte specific primer extension (ASPE).
d) Hybridization of the ASPE primers to a microarray followed by washing.
e) Scanning of the microarray.
f) Signal detection and analysis.

Steps (c) through (f) are automated by the CE marked INFINITI Analyzer or INFINITI PLUS Analyzer.

A schematic overview of the assay is shown below.



AutoGenomics

DEVICE DESCRIPTION

The INFINITI CYP450 2D6I Assay is an *in vitro* diagnostic device which utilizes AutoGenomics' proprietary film-based microarray technology combined with process automation, reagent management and software technology for the detection and genotyping of the CYP2D6 allelic variants in genomic deoxyribonucleic acid (DNA) obtained from whole blood samples.

The INFINITI CYP450 2D6I Assay is comprised of the BioFilmChip® Microarray and the Intellipac® Reagent Module.

The **BioFilmChip Microarray** consists of a polyester film coated with proprietary multi-layer components designed for DNA analysis. The layers have been designed to provide a versatile surface to enhance test performance. The microarrays are designed to be assay specific. The INFINITI CYP450 2D6I Assay uses a microarray chip (P-Chip) which contains unused capture probes which could potentially be used for certain specific assays. Therefore, multiple assays can be developed using the same microarray.

The **Intellipac Reagent Module** which acts as a communication link contains up to four reservoirs that house the test reagents and has an integrated memory chip. Information on the reagent such as lot number, expiration date and number of tests is archived in the memory chip.

The INFINITI CYP450 2D6I Assay should be run using the AutoGenomics **INFINITI Analyzer or INFINITI PLUS Analyzer**. The Analyzers are instruments used for clinical multiplex systems intended to measure and sort multiple signals from a clinical sample. The Analyzers are designed to measure fluorescence signals of labeled DNA target hybridized to BioFilmChip microarrays. The Analyzers automate the CYP450 2D6I Assay and integrates all the discrete processes of sample (PCR amplicon) handling, reagent management, hybridization, detection, and results analysis. The assays are processed automatically and the spots are read by the built-in confocal microscope. Results are analyzed and presented as genotype calls.

The INFINITI Analyzer and INFINITI PLUS Analyzer are CE marked.

Instructions on how to use the Analyzers are provided in the Operator's Manuals.

WARNINGS AND PRECAUTIONS

Handling Requirements

- **For *in vitro* diagnostic use. To be used by qualified laboratory personnel.**
- This test is to be used only with whole blood collected in EDTA. Do not freeze/thaw blood samples. Specimens should be assayed as soon as possible.
- All patient specimens are potentially hazardous and care should be taken when handling materials of human origin. No test method can offer complete assurance that HCV, HIV or other infectious agents are absent.
  Follow the CLSI Guidelines (Molecular Diagnostics Methods for Infectious Diseases; Approved Guidelines; MM3-A).
- Upon receipt of samples, visually inspect sample condition. Specifically, look for abnormal signs that indicate that sample integrity has been compromised (e.g., evaporation, decrease in volume, precipitation, spills, discoloration, sedimentation, separation, turbidity, etc.). If you observe or suspect any sample abnormality, do not perform any test.
- Samples should be handled with extreme caution to prevent contamination, spillage, sample mix-up. Sample containers should be labeled clearly to prevent mix-up.
- Store samples at the specified conditions.
- To minimize the risk of cross contamination, sample preparation, PCR reaction set up and PCR product analysis should be performed according to approved guidelines such as CLSI (Molecular Diagnostic Methods for Genetic Diseases: Approved Guideline).
- Do not pool/mix reagents from different lots.

- Do not use a kit or reagent past its expiration date.
- Store kits and reagents according to the product label.

Laboratory Procedures
- Follow normal precautions for handling laboratory reagents.
- Follow safe laboratory procedures: do not pipette by mouth; wear protective clothing (e.g., disposable gloves and laboratory coats) and eye protection; do not eat, drink or smoke in the laboratory work areas; wash hands thoroughly after handling samples and reagents.

Waste Handling
- Dispose of unused reagents, specimens and waste according to applicable country, federal, state and local regulations.
- Material Safety data Sheets (MSDS) are available upon request from AutoGenomics Customer Service.

Sample Preparation
- Refer to the safety instructions in the package insert provided with the DNA extraction kit used.
- The PCR product can not be stored prior to loading it onto the microarray. Use immediately**.**

INFINITI Analyzer and INFINITI PLUS Analyzer
- **Read the Operator's Manuals before operating the instruments.** Pay particular attention to "Notes".
- Follow the Caution and Safety Warning in the Operator's Manual.
- Refer to the Installation Requirements Section when installing the instrument.
- Refer to the Errors Section when errors are encountered while operating the instrument.
- Refer to the Help Section when problems are encountered.

STORAGE / STABILITY

BioFilmChip Microarray: 12 months Refrigerated (2 to 8°C)

Intellipac Reagent: 12 months Refrigerated (2 to 8°C)

Note: Remove the Intellipac from the Analyzer and store refrigerated as soon as possible. Do not use after Intellipac has been opened for four weeks.

Amplification Mix: 18 months Frozen (-30 to -15°C)

Note: Specific product expiration date is printed on the product label.

SPECIMEN COLLECTION AND STABILITY
- Peripheral blood drawn in an EDTA (purple-top) tube.
- Do not freeze / thaw blood samples. Specimens should be assayed as soon as possible.

MATERIALS PROVIDED (SUFFICIENT FOR 48 TESTS)
- Product Number 03-1110-02 INFINITI CYP450 2D6I BioFilmChip® Microarray Magazine
- Product Number 03-2110-02 INFINITI CYP450 2D6I Intellipac® Reagent Module

  24 tests per module which contains:

  1.1 ml ASPE Master Mix:
  - dNTPs
  - Labeled-dCTP
  - Allele Specific Primers
  - Extension Reaction Buffer

  2.6 ml Hybridization Buffer
  - SSC
  - Hybridization Positive Control
  - Sodium Azide Preservative 0.08%

- Product Number 03-3110-02 CYP450 2D6I Amplification Mix
  4 x 250µl vials of PCR reaction master mix containing:
  dNTPs
  PCR Primer Mix
  $MgCl_2$
  PCR Reaction Buffer
- **FOR INFINITI Analyzer***:* Product Number 12-0010-00: Wash buffer
  OR
  FOR INFINITI PLUS Analyzer*:* Product Number 12-0330-00: Buffer Solution BF1

REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- DNA Extraction Kits - The INFINITI CYP450 2D6I Assay can detect the CYP450 2D6 allelic mutations using genomic DNA isolated from blood with sufficient purity, i.e., with the ratio of absorbance at 260 nm to absorbance at 280 nm of ≥ 1.60, and a concentration of approximately 20 ng DNA/µl. Any DNA extraction method that meets this specification may be used. The INFINITI CYP450 2D6I Assay has been tested with several commercially available kits. The user can contact AutoGenomics for further information.
- Distilled Water (DNAse and RNAse free)
- Wash Buffer (Catalog # 12-001)
- Platinum Taq DNA Polymerase (Invitrogen, Catalog No:10966-018)
- Titanium Taq DNA Polymerase (Clontech Catalog No: 639209)
- Shrimp Alkaline Phosphatase (SAP, USB, product No: 70092Z)
- Exonuclease I (EXO I, USB, product No: 70073X)
- Distilled water (DNAse and RNAse free)

EQUIPMENT

The following equipment is required but not provided with the assay reagents

- Pipettors
- Mini Centrifuge
- Pipette tips
- Microfuge tube Racks
- Thermocycler
- Vortex
- 0.2 ml thin wall tubes for PCR
- 1.5 ml microcentrifuge tubes
- 8-well Flat Strip Caps (Genesee Scientific, Catalog No. 22-623)
- AutoGenomics Product Number 11-0060-00: INFINITI Waste tray Stir Bars
- AutoGenomics Product Number 11-0020-00: INFINITI Waste Tray Liners
- AutoGenomics Product Number 11-0080-00: INFINITI Pipette Tips

- **FOR INFINITI Analyzer***:*
  - AutoGenomics Product Number 10-0010-99: INFINITI Analyzer
  - AutoGenomics Product Number 11-0030-00: 24-Well Plates with Lids
  - AutoGenomics Product Number 11-0050-00: INFINITI Temp Cycle Plate

- **FOR INFINITI PLUS Analyzer***:*
  - AutoGenomics Product Number 10-0020-99: INFINITI PLUS Analyzer
  - AutoGenomics Product Number 11-0100-00: 48-Well Plates
  - AutoGenomics Product Number 11-0110-00: 48 Well Plate Lid (reusable)

RECOMMENDED DNA CONTROLS

It is recommended that positive controls (heterozygous and/or homozygous samples) be included in each test run. In addition, a negative control (i.e., wild type sample) and a no template control (i.e., molecular grade water) should also be included in each test run. Coriell DNA samples (www.coriell.org) are suitable positive controls for many of the detected genotypes. Please contact AutoGenomics for recommendations on use of Coriell DNA.

ASSAY PROCEDURE

DNA Extraction

Follow the instructions provided with the DNA extraction kit used.

PCR Reaction

Note:

- Keep Taq DNA polymerase on ice.
- Completely thaw reagents on ice.
- Vortex the amplification mix tube for 2 to 5 seconds then centrifuge briefly to bring the contents to the bottom of the tube.
- To avoid contamination, a separate area is recommended for assembly of the PCR reaction. Decontaminate pipettes and all work surfaces with freshly prepared 10% bleach in deionized or distilled water.
- Filter tips and gloves must be used when handling specimens and controls.
- The PCR product cannot be stored prior to testing. Use immediately.

Note:

- For the INFINTI Analyzer use the 24WP.
- For the INFINTI PLUS Analyzer use the 48WP.

1. Prepare the PCR master mix for **reaction A**

Amplification mix	16.85 µl
Platinum Taq polymerase	0.15 µl
Total volume of PCR Master mix	17.0 µl

Note: Calculate the amount of each reagent needed based on the number of reactions.

2. Gently vortex the PCR master mix then dispense 17 µl of master mix into wells of the well plate.
3. Add 3 µl of sample DNA (approximately 25 ng/µl) to each well.

PCR master mix	17.0 µl
Sample DNA	3.0 µl
Total volume of amplification reaction	20.0 µl

4. Prepare the PCR master mix for **reaction B**

Amplification mix	16.85 µl
Platinum Taq polymerase	0.15 µl
Total volume of PCR Master mix	17.0 µl

Note: Calculate the amount of each reagent needed based on the number of reactions.

5. Gently vortex the PCR master mix then dispense 17 µl of master mix into wells of the well plate.
6. Add 3 µl of sample DNA (approximately 25 ng/µl) to each well.

PCR master mix	17.0 µl
Sample DNA	3.0 µl
Total volume of amplification reaction	20.0 µl

7. Place the well plate, sealed with 8-well flat strip caps, in a thermocycler and immediately commence the amplification reaction using the following program.

Step No.	Temperature	Time	No. of Cycles
1	94	2 min.	N/A
2	94 66-60 (-0.3°C/cycle) 68	15 sec. 30 sec. 4 min.	20x
3	94 58 68	15 sec. 30 sec. 4 min.	15x
4	68 4	1 min. Hold	N/A

Note: After each cycle in step 2 the temperature is decreased by 0.3°C. When an Eppendorf Mastercycler EP was used with the ramp rate set at 100%, the total cycling time was 3 hours and 10 minutes (± 5 min). If using other thermocycler models we recommend adjusting the ramp rate in order to obtain an equivalent total cycling time.

SAP and Exonuclease I Treatment

Post PCR clean up is a critical step to ensure the remaining substrates would not carry through and interfere with the signal amplification. Prepare the enzymes mixture as a master mix. For example, if there are 9 PCR reactions, create a master mix enough for 10 reactions by pipetting 30 µl of SAP, 7.5 µl of Exo, and 2.5 µl of Titanium Taq.

SAP	3.00 µl
Exonuclease I	0.75 µl
Titanium Taq	0.25 µl
Total	4.00 µl

Dispense 4 µl of the enzyme mix per reaction into a clean well plate. Dispense 10 µl from the completed PCR reaction A and 10 µl from completed PCR reaction B (of the same sample) to the 4 µl enzyme mix in the specified well.

Sample Loading

1) Carefully remove the 8-well flat strip caps to avoid splashing.
2) Load the well plate in the appropriate orientation (with well A1 in the back left corner) into the Analyzer
   - **INFINITI Analyzer:** Load the assembled 24WP with the associated lid (Catalog # 11-0030-00).
   - **INFINITI PLUS Analyzer:** Load the assembled 48WP with a clean 48WP lid (see instructions in the INFINITI PLUS Analyzer Operator's Manual) (Catalog # 11-0110-00, reusable).

3) Load the following: assay specific magazines, Intellipac, INFINITI Static Free Pipette tips, and buffer.
   - **FOR INFINITI Analyzer***:*
     Wash Buffer should be placed in the INFINITI bottle holders. The Wash Buffer goes in the left holder (near the magazine).
   - **FOR INFINITI PLUS nalyzer***:*
     Follow the INFINITI PLUS Analyzer Operator's Manual for checking and replacing Buffer solution BF1.

Operation of the Analyzers

Follow the instructions in the Operator's Manuals

INFINITI Analyzer Operator's Manual (Part Number EM-34000)
INFINITI PLUS Analyzer Operator's Manual (Part Number EM-34041)

QUALITY CONTROL

- Maintain calibration of thermocycler according to manufacturer's specifications.
- Maintain calibration of INFINITI or INFINITI PLUS Analyzer according to AutoGenomics' specifications.
- Maintain calibration of pipettes according to manufacturer's specifications.

It is recommended that positive controls (heterozygous and/or homozygous samples) be included in each test run. In addition, a negative control (i.e., wild type sample) and a no template control (i.e., molecular grade water) should also be included in each test run. Coriell DNA samples (www.coriell.org) are suitable positive controls for many of the detected genotypes. Please contact AutoGenomics for recommendations on use of Coriell DNA.

Note: The thermal cycler used should be regularly maintained and calibrated with an external temperature standard, according to the laboratory's regulatory and QC requirements.

LIMITATIONS

The results obtained from the INFINITI CYP450 2D6I Assay should be used and interpreted only in the context of the overall clinical diagnosis. AutoGenomics is not responsible for any clinical decisions that are taken.

INTERPRETATION OF RESULTS

The INFINITI CYP450 2D6I Assay is designed to detect and genotype CYP450 2D6 mutations. The assay results are provided as a genotype "call", indicating which genotype was detected in the sample, i.e.,

W	=	Wild
M	=	Mutant
H	=	Heterozygous
P	=	Positive
N	=	Negative

When the assay is not completed, and no genotype call is made, the assay will need to be repeated. The report displays a message which indicates the reason why no genotype call was made. When an error occurs (e.g., "low DNA"), an Error Log is generated which identifies the problem. Please refer to the Trouble Shooting section of the INFINITI Analyzer Operator's Manual.

PERFORMANCE CHARACTERISTICS

Analytical Specificity

Studies related to specificity were conducted during assay development. There are three levels of specificity required in the assay:

- PCR primers
- ASP – target binding region
- ASP – chip binding region

PCR primer specificity was determined by amplicon size on a gel and sequencing the amplicon. ASP primer specificity was determined by the correct calls made by the assay using known samples (e.g., Coriell). Capture probe specificity is determined by hybridizing different oligos and demonstrating that only the correct oligo hybridizes to the known spot.

Limit of Detection (LoD)

The analytical sensitivity (Limit of Detection) of the INFINITI CYP450 2D6I Assay was assessed by analysis of two (2) blood samples, representing five (5) alleles, at concentrations of 150, 25, and 10 ng DNA input per test and using the data from the reproducibility and interference studies which used eight (8) samples (representing seven alleles) at the target 60 ng DNA input level. The DNA was extracted using the Qiagen QIAMP DNA Kit, PSS Magtration System 12GC and Charge Switch. Eight (8) replicates of each sample were assayed at the 150, 25, and 10 ng DNA input level. At the target 60 ng input level, each sample was assayed 40 times for a total of 320 tests.

The Limit of Detection study demonstrated that the INFINITI CYP450 2D6I Assay can detect the target mutations at the recommended DNA input concentration of 20 ng DNA/µl. The assay requires 3 µl of the DNA sample for 60 ng DNA input per test.

Assay Precision and Reproducibility

Assay precision and reproducibility studies involved eight (8) whole blood samples, three operators, three instruments and three lots of reagents. Each operator received identical sets of the sample blood aliquots. The operators were blinded to the samples.

DNA was extracted from the whole blood samples as soon as received. Each operator used a different extraction method. Samples were assayed on five non-consecutive days by each operator.

The following provides a summary of the precision and reproducibility studies.

sample	genotype	samples tested	1st time run			
			samples with correct calls	samples with incorrect calls	samples with no call	correct call rate (%)
1	*4/ *10	40	37	0	3	92.5
2	*5 Del	40	38	0	2	95.0
3	*2/ *41	40	39	0	1	97.5
4	*2/*2, *41/*41	40	37	0	3	92.5
5	*2/*2, *2A/ *41	40	39	0	1	97.5
6	*1/ *10	40	39	0	1	97.5
7	*1/ *10	40	37	0	3	92.5
8	*1/ *1	40	39	0	1	97.5
Totals		320	305	0	15	95.3

Sample Carry-over
Sample carryover studies demonstrated that there is no sample carry-over with the INFINITI CYP450 2D6I Assay. Three (3) Coriell DNA samples were used; Water was used as "no template control".

Potential Interference from Drugs/Chemicals
Interference from potential interfering substances was evaluated using four (4) whole blood samples. The interference studies demonstrated that INFINITI CYP450 2D6I Assay performance was not affected by the addition of the following substances.

Substance Added	Concentration
Bilirubin, Conjugated	60 mg/dl
Bilirubin, Unconjugated	60 mg/dl
Triglycerides	3000 mg/dl
Human albumin	6 g/dl

Analytical Specificity – Method Comparison
The INFINITI CYP450 2D6I Assay was compared to the CE-marked Roche AmpliChip as the comparator method. Patient samples used during the studies were de-identified to protect patient's identity. A total of 131 alleles were tested. The results of the comparison studies demonstrated 94% agreement with the CE-marked Roche AmpliChip on first time test. There were no incorrect calls during the comparison studies. Eight (8) samples had to be repeated for "no calls". "No calls" were repeated once. Repeat tests agreed 100% with the Roche AmpliChip results.