AutoGenomics

INFINITI® BRAF Assay

Directional Package Insert (DPI)

For *In Vitro* Diagnostic Use

CE

FOR EXPORT ONLY

Manufactured by AutoGenomics, Inc., 1600 Faraday Avenue, Carlsbad, CA USA 92008

Authorized EU Agent: Medical Device Safety Service GmbH (MDSS)
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INTENDED USE

The INFINITI BRAF Assay is an *in vitro* diagnostic test for the detection and identification of the most prevalent BRAF amino acid changes in positions known to affect the function of the proteins. These mutations are identified by detecting their codon variants or nucleotide changes. The INFINITI BRAF assay is designed to detect these mutations in formalin-fixed and paraffin-embedded (FFPE) tissue samples.

The INFINITI BRAF Assay is a qualitative assay for use in clinical laboratories upon prescription by the attending physician.

BACKGROUND INFORMATION

BRAF

BRAF is a human gene that makes a protein called B-Raf. The B-Raf protein is involved in sending signals inside cells, which are involved in directing cell growth. In 2002, it was shown to be faulty (mutated) in human cancers. [1]

Acquired mutations in this gene have been found in cancers, including non-Hodgkin lymphoma, colorectal cancer, malignant melanoma, papillary thyroid carcinoma, non-small cell lung carcinoma, and adenocarcinoma of lung.

More than 30 mutations of the *BRAF* gene associated with human cancers have been identified. The frequency of BRAF mutations varies widely in human cancers from more than 80% in melanomas and nevi, to as little as 0-18% in other tumors, such as 1-3% in lung cancers and 5% in colorectal cancer.[10] In 90% of the cases, thymine is substituted with adenine at nucleotide 1799. This leads to valine (V) being substituted for by glutamate (E) at codon 600 (now referred to as V600E) in the activation segment that has been found in human cancers.[11] This mutation has been widely observed in papillary thyroid carcinoma, colorectal cancer, melanoma and non-small-cell lung cancer.[2][3][4][5][6][7][8]

TEST PRINCIPLE/ASSAY OVERVIEW

The INFINITI BRAF assay is designed to detect the most prevalent BRAF amino acid changes in positions known to affect the function of the proteins. These mutations are identified by detecting their codon variants or nucleotide changes.

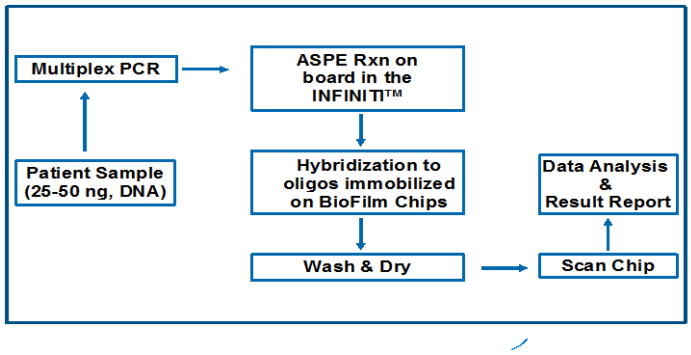
Gene	Codon	Analyte	Reported mutations detected		
BRAF	600	V600A	Val600Ala	c.1799T>C	GTG>GCG
		V600D	Val600Asp	c.1799_1800TG>AT	GTG>GAT
		V600E1	Val600Glu	c.1799T>A	GTG>GAG
		V600E2	Val600Glu	c.1799_1800TG>AA	GTG>GAA
		V600KRM	Val600Lys	c.1798_1799GT>AA	GTG>AAG
			Val600Arg	c.1798_1799GT>AG	GTG>AGG
			Val600Met	c.1798G>A	GTG>ATG

The assay protocol includes the following five major processes:

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

Steps (b) through (e) are automated by the CE marked INFINITI Analyzer or INFINITI PLUS Analyzer.

A schematic overview of the assay is shown below.



DEVICE DESCRIPTION

The INFINITI BRAF Assay utilizes AutoGenomics' proprietary film-based microarray technology combined with process automation, reagent management and software technology for multiplex detection of the most prevalent BRAF amino acid changes in positions known to affect the function of the proteins. These mutations are identified by detecting their codon variants or nucleotide changes.

The INFINITI BRAF Assay is comprised of the BioFilmChip® Microarray, the Intellipac® Reagent Module and the PCR Amplification Mix.

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 INFINITI BRAF Assay uses a microarray chip which has capture probes spotted on the surface of the film. One sample can be run on one microarray. Twelve (12) microarrays are housed in a magazine.

The **Intellipac Reagent Module** which acts as a communication link contains four reservoirs that house the test reagents and has an integrated memory chip. Reagent information such as lot number, expiration date, and volume usage are stored in the memory chip. The Intellipac Reagent Module communicates with the INFINITI Analyzer and provides the reagent information which appears on the assay report and printout. The Intellipac Reagent Management Module provides test reagent for 24 samples.

The **PCR Amplification Mix** consists of the reagents needed for the PCR amplification step of the assay. Each box of the PCR Amplification Mix provides 4 x 250µl vials of PCR Amplification.

The **INFINITI Analyzer** and **INFINITI PLUS Analyzer** automates the INFINITI BRAF Assay and integrates all the discrete processes of sample (PCR reaction product) handling, reagent management, hybridization, detection, and results analysis. The assays are processed automatically and read by the built-in confocal microscope. Results are analyzed and presented as positive or negative for the presence of the mutations.

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

The INFINITI Analyzer and INFINITI PLUS Analyzer are CE marked.

WARNINGS AND PRECAUTIONS

Handling Requirements

- **For *in vitro* diagnostic use. To be used by qualified laboratory personnel.**
- 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.
- Safety data Sheets (SDS) 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 cannot 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: 24 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

MATERIALS PROVIDED (SUFFICIENT FOR 48 TESTS)

- Catalog Number 02-1120-02 INFINITI BRAF BioFilmChip® Microarray Magazine
- Catalog Number 02-2120-02 INFINITI BRAF 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%
- Catalog Number 02-3120-02 INFINITI BRAF Amplification Mix
  4 x 250µl vials of PCR reaction master mix containing:
  dNTPs
  PCR Primer Mix
  $MgCl_2$
  PCR Reaction Buffer
- Product Number 12-0010-02: Wash buffer

REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- DNA Extraction Kits - The INFINITI BRAF Assay can detect the target BRAF mutations using genomic DNA, isolated formalin-fixed and paraffin-embedded (FFPE) tissue samples, 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 15ng DNA/µl. Any DNA extraction method that meets this specification may be used. The INFINITI BRAF Assay has been tested with several commercially available kits. The user can contact AutoGenomics for further information.
- Titanium Taq DNA Polymerase (Clontech, Catalog No: 639209)
- Exonuclease I (USB, Catalog No: 70073)
- Shrimp Alkaline Phosphatase (USB, Catalog No: 70092)
- Distilled water (DNAse and RNAse free)

EQUIPMENT

The following equipment are 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)

DNA CONTROLS

It is required to run known positive controls and a negative control should also be included in each test run.. Cell lines or commercially available DNA with known BRAF mutations can be used as positive controls (www.atcc.org). If cell lines are acquired, they will have to be cultured and then the genomic DNA isolated. Please contact technical support at AutoGenomics if additional information is required.

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. Thaw Amp mix on ice, centrifuge briefly, vortex 2 to 5 seconds and centrifuge briefly.

2. Prepare the PCR master mix.

PCR reaction master mix	17.8 µl

Titanium Taq DNA polymerase	0.2 µl
Total volume of PCR master mix	18.0 µl

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

3. Gently vortex the PCR master mix then dispense 18 µl of master mix into wells of the well plate.

4. Add 2 µl of sample DNA to each well.

PCR master mix	18.0 µl
Sample DNA	2.0 µl
Total volume of amplification reaction	20.0 µl

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

Step No.	Temperature °C	Time (sec)	No. of Cycles
1	94	120	1
2	94 67 – 57 (-1.0/cycle)	15 15	10
3	94 57	15 15	30
4	94 4	15 Hold	1

Note: After each cycle in step 2 the temperature is decreased by 1.0°C. When an Eppendorf Mastercycler EP was used with the ramp rate set at 75%, the total cycling time was 1 hour and 4 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.

1. 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 15µl of SAP, 3.75µl of Exonuclease, and 1.25µl of Titanium Taq.

   Enzymes mixture (per reaction):

SAP	1.500 µl
Exonuclease I	0.375 µl
Titanium Taq	0.125 µl
Total volume of SAP/Exo reaction	2.000 µl

2. Vortex 2 to 5 seconds and centrifuge briefly.

3. Dispense 2 µl of the enzyme mixture per reaction into the completed PCR reaction, seal with 8 well strip caps.

4. Vortex for 1 to 2 seconds and centrifuge briefly.

5. Incubate in thermal cycler using the following conditions.

Step No.	Temperature °C	Time (min)
1	37	60
2	94	20
3	4	Hold

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 Analyzer***:*
     Follow the INFINITI PLUS Analyzer Operator's Manual for checking and replacing Wash Buffer.

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.

LIMITATIONS

The results obtained from the INFINITI BRAF 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

When the integrity of a sample is in question because of suboptimal DNA quantity and/or quality, an amplicon could fail to meet the software's minimum threshold for mutation detection resulting in blanked out calls. In this case, an asterisk shows in the analysis column and the statement: "Calls are not made for amplicons which do not meet the minimum requirements for mutation detection" is printed at the bottom of the results page. Under these circumstances, a sample may need to be repeated using more (up to 4µl) of DNA in the PCR reaction.

If there are unexpected multiple positives within the same exon from a DNA sample, then chances are insufficient PCR product was made, or assay performance was suboptimal. In this case, the assay should be repeated for that sample.

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., ATCC strain). 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)

A limit of detection study was conducted to demonstrate the optimal detection range for the INFINITI BRAF Assay. A known DNA sample (HTB-38D positive for V600E1) was diluted to 30ng, 15ng and 5ng. This was equivalent to 60, 30 and 10ng per test. The target/recommended DNA input is 30ng per test. Eight (8) replicates of each sample dilution were tested.

All 24 tests for HTB-38D were positive for V600E1. The study demonstrated that the optimal detection range for the INFINITI BRAF Assay was 10 to 60ng DNA input per test.

Reproducibility

A study was performed to demonstrate the performance of the assay using different lots of reagents, different instruments and different operators and on different days. The study was performed to demonstrate that the INFINITI BRAF reagents can be manufactured that consistently meet the assay specifications and give the correct genotype calls.

The reproducibility data represented six (6) Intellipac reagent lots, five (5) lots of AMP Mix, six (6) lots of microarray chips and four (4) INFINITI Analyzers. The tests were run by three (3) operators and on different days. The same known DNA sample (positive for V600E1) was tested. All tests gave the correct calls.

The data demonstrated that the INFINITI BRAF Assay reagents can be manufactured which consistently meet the specifications of the assay.

Sample Carry-over

Sample carryover studies were performed using ATCC cell lines (HTB-26D and HTB-38D) and a Coriell DNA sample. HTB-26D (positive for G13D), HTB-38D (positive for V600E1), and NA 17017 (wild type sample) were run alternatively at different concentrations to determine if one sample will carry over to next. Results of the studies indicated no sample carry-over contamination.

Potential Interference from Drugs/Chemicals
KRAS and BRAF assays that are commercially available use the same specimens and extraction methods and have demonstrated that the quality of the DNA is not affected by any potential interfering substance.

Analytical Specificity – Method Comparison
Clinical validation studies were conducted to validate the INFINITI BRAF Assay results by sequencing or by a test method currently in use by the laboratory (comparator method). The following were the requirements for the clinical studies:

- Specimens: formalin-fixed and paraffin-embedded (FFPE) tissue samples
- Extraction method: QIAmp DNA Kit
- Concentration of extracted DNA: 15ng/µl
- Acceptance criteria: ≥ 90% concordance with comparator methods

Clinical validation study for the BRAF mutation detection was done using actual clinical data. The INFINITI BRAF Assay detected 93.5% (43/46) correct calls and 2.2% (1/46) incorrect calls compared to the comparator method, and had 4.3% (2/46) incorrect calls.

REFERENCES

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