IntelliPlex™ BRAF V600 Mutation Kit User Manual

REF 82004 24 Reactions

CE IVD For In-Vitro Diagnostic Use

PlexBio Co., Ltd.
6F-1, No. 351, Yangguang St., Neihu Dist.
Taipei City, 11491, Taiwan
TEL: + 886-2-2627-5878
http://www.plexbio.com

European Authorized Representative:
EC REP Medical Device Saftey Services GmbH (MDSS)
Schiffgraben 41, 30175, Hannover, Germany

IMPORTANT:
Read the instructions carefully prior to use

1. INTENDED USE

The IntelliPlex BRAF V600 Mutation Kit, based on πCode™ technology and PlexBio's instrument platform, is an in-vitro molecular assay intended for qualitative identification of 7 nucleotide changes on exon 15 of the BRAF gene using DNA samples derived from formalin-fixed paraffin-embedded (FFPE) human tissue, such as melanoma, non-small cell lung cancer (NSCLC) or metastatic colorectal cancer (mCRC). The IntelliPlex BRAF V600 Mutation Kit is intended to assist clinicians in identifying patients who may benefit from BRAF targeted treatment using Vemurafenib.

2. INTRODUCTION

BRAF encodes a serine-threonine protein kinase called B-Raf which is composed of three conserved domains: a Ras-GTP-binding self-regulatory domain, a serine-rich hinge domain, and a catalytic protein kinase domain. During the signal transduction, Ras-GTP binds and activates B-Raf to phosphorylate other signal transduction molecules such as MEK, leading to cellular proliferation. Many types of cancers such as melanoma, NSCLC or mCRC are associated with mutations in the BRAF gene which are often found in exon 15 on codon 600. Such mutations can activate signal transduction in pathways leading to increased cellular proliferation in the absence of EGFR activation.

Vemurafenib is a selective oral inhibitor of the BRAF serine-threonine kinase and is demonstrated for the efficacy treatment among metastatic melanoma patients with BRAF V600 mutations. Although Vemurafenib used in non-melanoma cancers has not been systematically explored, the therapeutic potential is still considerable.

It is thus critical to assess the mutation status of the BRAF gene. Detection of 7 mutations of the BRAF gene in a single reaction from specimens with large amount of wild-type genomic DNA is feasible based on the SelectAmp and πCode technology. The identification of 7 nucleotide changes on exon 15 of the BRAF gene are listed in Table 1.

Table 1: Mutations of the BRAF gene

Exon	Amino Acid Change	Nucleotide Change
15	V600E1	c.1799T>A
	V600E2	c.1799_1800TG>AA
	V600D	c.1799_1800TG>AT
	V600G	c.1799T>G
	V600K	c.1798_1799GT>AA
	V600R	c.1798_1799GT>AG
	V600M	c.1798G>A

3. TECHNOLOGICAL PRINCIPLES

The IntelliPlex BRAF V600 Mutation Kit utilizes two technologies- SelectAmp and πCode - for detection of 7 BRAF gene mutations in one reaction.

SelectAmp-

Mutation-specific multiplex PCR amplification is achieved by SelectAmp technology, which uses the Locked Nucleic Acid (LNA) to block the amplification of the wild-type sequence. Thus, a specific mutant sequence can be selectively amplified and dramatically increases the sensitivity and the specificity.

πCode MicroDisc-

πCode MicroDisc is manufactured to generate up to 16,000 distinct circular image patterns for multiplexing applications. Each πCode has a distinct circular image pattern, which corresponds to a specific capture agent conjugated to the surface of the disc. All capture agent

tagged πCode are pooled, enabling capturing and detection of specific analytes in one reaction.

Detection Principle

The test is based on five processes listed as follows:

I. DNA extraction from FFPE specimens
II. Mutation-specific multiplex PCR amplification
III. Hybridization of PCR amplicons with mutation-specific probes tagged πCode in one well reaction
IV. Incubation with SA-PE for fluorescent labelling
V. Image pattern decoding and fluorescent signal detection by the PlexBio™ 100 Fluorescent Analyzer

4. WARNINGS AND PRECAUTIONS

- For in-vitro diagnostic use.
- This assay kit should be used by qualified laboratory personnel only.
- Do not use a kit or reagent past its expiration date.
- Note that tumor samples are non-homogeneous and may also contain non-tumor sections from a same sample to cause false-negative results.
- Component reagents have been diluted optimally. Further dilution of the component reagents is not recommended.
- Specimens should be handled as infectious material. Please follow universal precaution for safe use.
- Store assay kits and reagents according to the product label and instructions.
- Do not mix reagents from different lots.
- Dispose of unused reagents, specimens and waste according to applicable central/federal, state, and local regulations.
- Wear powderless gloves and do not touch and make any markings on the bottom of the plate at any time, as fingerprints and markings would interfere with decoding and signal acquisition.
- General laboratory precautions should be taken:
  - Do not pipette by mouth.
  - Wear protective clothing (e.g., disposable powderless gloves and laboratory coats) and eye protection.
  - Do not eat, drink or smoke in the laboratory.
  - Wash hands thoroughly after handling samples and reagents.
- The workspace including racks and pipettes should be thoroughly cleaned and wiped with 0.5% sodium hypochlorite solution followed by wiping with a 70% ethanol solution. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.
- Material Safety Data Sheets (MSDS) are available upon request from PlexBio Customer Service.

5. KIT COMPONENTS

The IntelliPlex BRAF V600 Mutation Kit contains sufficient reagents for up to 24 tests. The kit components supplied are listed as followings.

1. BRAF Reaction Mix
Ref. No.: 20097
Quantity & Volume: 1 vial, 264 µL/vial
Description: For PCR amplification
Contents: 36.4% MyFi 5X Reaction Buffer
Magnesium chloride
dNTPs and Enhancer
3.6% MyFi DNA polymerase (Microbial)

2. BRAF Primer Mix
Ref. No.: 20098
Quantity & Volume: 1 vial, 120 µL/vial
Description: For PCR amplification
Contents: <0.01% Forward Primer
<0.01% Reverse Primer
<0.1% Locked Nucleic Acid
Internal Control Plasmid DNA

3. BRAF πCode MicroDisc
Ref. No.: 20099
Quantity & Volume: 1 vial, 480 µL/vial
Description: For PCR amplicon capture
Contents: Glycerol
Phosphate buffered saline
0.1% Albumin, from bovine (Biological)
<0.1% EDTA
<0.1% Sodium azide

4. BRAF POS Control
Ref. No.: 20101
Quantity & Volume: 1 vial, 20 µL/vial
Description: Assay positive control
Contents: <0.001% BRAF plasmid DNA (Microbial)
Tris-EDTA Buffer

5. BRAF NEG Control
Ref. No.: 20102
Quantity & Volume: 1 vial, 20 µL/vial
Description: Assay negative control
Contents: ddH2O

6. SA-PE Solution
Ref. No.: 20007
Quantity & Volume: 1 bottle, 7 mL/bottle
Description: Streptavidin-phycoerythrin for fluorescent signal acquisition
Contents: Phosphate buffered saline
0.5% Streptavidin-phycoerythrin
1% Albumin, from bovine
<0.1% Sodium azide

7. **BRAF Hy Buffer**
   Ref. No.: 20100
   Quantity & Volume: 1 bottle, 2.4 mL/bottle
   Description: For hybridization
   Contents: Saline-Sodium Phosphate-EDTA

8. **BRAF 10X Wash Buffer**
   Ref. No.: 20104
   Quantity & Volume: 1 bottle, 50 mL/bottle
   Description: For πCode washing
   Contents: Phosphate buffered saline
   1% Tween-20
   <0.1% Sodium azide

NOTE: POS Control, NEG Control and Hy Buffer stand for positive control, negative control and hybridization buffer, respectively.

6. MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- 96-well plate (Greiner Bio-one; Cat. No. 655101)
- Clean tubes for PCR reaction
- Dedicated micropipette
- Filter tips for micropipette
- ddH_2O for dilution of 10X Wash Buffer
- FFPE DNA extraction kit (QIAamp DNA FFPE Tissue Kit, Qiagen; Cat. No. 56404)
- Vortex mixer
- Microcentrifuge
- U Tray (PlexBio; Cat. No. 80023)
- V Tray (PlexBio; Cat. No. 80024)
- DigiPlex™ Thermocycler (PlexBio; Cat. No. 80018)
- IntelliPlex 1000 πCode Processor (PlexBio; Cat. No. 80033)
- PlexBio 100 Fluorescent Analyzer (PlexBio; Cat. No. 80000)
- Industrial Computer (PlexBio; Cat. No. 80002)
- DeXipher™ MD (PlexBio; Cat. No. 80051)

7. STORAGE, STABILITY AND TRANSPORTATION

Storage

Please store the IntelliPlex BRAF V600 Mutation Kit at 2°C to 8°C. Once opened, the reagents are stable for 3 months or until the expiration date, whichever comes first.

Stability

Do not use the IntelliPlex BRAF V600 Mutation Kit when it is expired. All components are stable up to the expiration date on the label if handled and stored under the recommended conditions.

Transportation

The shipping temperature for the IntelliPlex BRAF V600 Mutation Kit is at 2-8°C. If the kit is broken or has components missing, please contact the PlexBio Customer Service.

8. INSTRUMENT AND SOFTWARE

Instrument

Refer to the instrument user manual for complete installation and operation instructions (Thermocycler, IntelliPlex 1000 πCode Processor and PlexBio 100 Fluorescent Analyzer).

Software Installation

NOTE:

- ***For the first time assay operation, please make sure the KIT APP is installed into DeXipher.***
- ***The ENC file contains the information of kit lot no. and expiration date.***

KIT APP Installation

1. Open the BRAF KIT APP from the USB drive provided and run “Installer.exe”.
2. Click on “Install” from the pop up window and finish the APP installation.

Note: The KIT APP only needs to be installed for the first time running until the new version release.

ENC File Installation

1. Download the corresponding ENC File from PlexBio’s website (Homepage/ Download/ ENC Files/ BRAF Kit/ Corresponding Kit Lot no.)
2. Save to the computer and make sure the ENC file matches the lot no. of the assay kit.
3. Click on the button as shown below on the DeXipher homepage.

4. Click on the button as shown below to import kit.

5. Select and import the corresponding ENC file into the software.

9. SPECIMENS

Specimen Collection

The formalin-fixed paraffin embedded (FFPE) tissues have been validated to use with the IntelliPlex BRAF V600 Mutation Kit. It is recommend to use the QIAamp DNA FFPE Tissue Kit (50) (Qiagen; Cat. No. 56404) for DNA extraction.

Specimen Transportation and Storage

FFPE specimens can be transported and stored over 12 months at 15-30°C.

Storage of Extracted DNA

Extracted DNA can be stored at 2°C to 8°C for immediately use, or at -15°C to -25°C for long-term storage. Do not repeatedly freeze and thaw the extracted DNA.

10. ASSAY PROCEDURE

Warning:
Read the instructions carefully and follow every step of the assay protocol correctly.

10.1 DNA Extraction

1. Follow the instructions provided by the DNA extraction kit manufacturer. It is recommended to use QIAamp DNA FFPE Tissue Kit for FFPE section specimens with the elution volume of 50 µL.
2. Quantify the DNA using a Nanodrop UV-Vis Spectrophotometer or Qubit Fluorometer according to the manufacturer's protocol.
3. The DNA Stock concentration from the specimens must be ≥ 2.5 ng/µL to perform the IntelliPlex BRAF V600 Mutation Kit. Each amplification per specimen is run by using 4 µL of a 2.5 ng/µL DNA Stock (total of 10 ng DNA).

10.2 Multiplex PCR Amplification

1. Vortex mix each sample before use.
2. Spin down and keep samples on ice.
3. Prepare PCR Reaction as follows for each sample:

Table 2: PCR Reaction Preparation*

Material	Vol. (µL) per reaction
BRAF Reaction Mix	11
BRAF Primer Mix	5
Extracted DNA/PC/NC	4
Total	20

***Note:**

- *The amount of Reaction Mix and Primer Mix required depends on the number of reactions.*
- *Both POS Control and NEG Control reactions should be included in every run of the assay.*

4. Mix by tapping the tubes and spin down before placing the tubes on the thermocycler. Set up the PCR program conditions as shown in Table 3:

Table 3: PCR Program Conditions#

Temp. (˚C)	Time	Cycles
95	5 min	1
95	20 sec	36
70	20 sec	
60	60 sec	
4	Hold	1

#*Note: Ramp rate: 1°C/sec*

10.3 DNA Hybridization and SA-PE Reaction

1. Transfer the 10X Wash Buffer to 1L Wash Buffer bottle of the PlexBio Wash Station or IntelliPlex 1000 πCode Processor supplied and add 450 ml ddH2O to prepare 1X Wash Buffer for use.
2. Pipet the desired volume of SA-PE solution into SA-PE solution tank (V-tray). Please note that the dead volume of V-tray is **500 µL** and the minimum usage of SA-PE is **one row**.

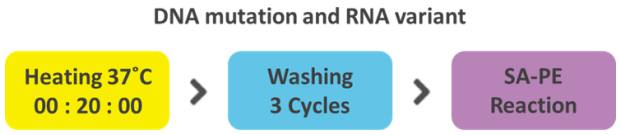
Calculation Example:
For 3 rows reaction, the SA-PE solution needed is **400 µL x 3 rows + 500 µL = 1.7mL (at least)**.
In order to ensure the instrument has sufficient solution to dispense, pipet extra volume into the solution tank is recommended.

***Note:**

- *SA-PE solution should be kept in the dark.*
- ***Do not** reuse the leftover of SAPE solution and the V-tray tank.*

3. Mix by vortexing the BRAF πCode MicroDisc for 10 seconds, and add 20 µL of BRAF πCode MicroDisc to each well directly without further pipetting. Vortex the BRAF πCode MicroDisc every 4 wells to ensure homogeneous suspension of the BRAF πCode MicroDisc.
4. Add 100 µL of BRAF Hy Buffer to each well.
5. Spin down the PCR products.
6. Denature the PCR products on the thermocycler by heating up to 95˚C for 5 minutes then cooling down to 4˚C without delay.

7. Spin down the PCR products, and keep PCR products on the ice before adding to wells.
8. Add 10 µL of the denatured PCR products to each well.
9. Refer to IntelliPlex 1000 πCode Processor operation manual and follow the instructions for built-in assay program as described (Homepage/ Molecular Assay/ Select well rows/ DNA&RNA/ Confirm procedure conditions as figure shown below/ Start Running). The plate will be ready for decoding once program finished.

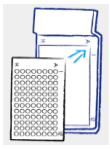


***Note:**

- ***Do not*** *open the door during the instrument operation.*

10.4 Image Decoding and Fluorescent Detection

1. Place the plate on the PlexBio 100 Fluorescent Analyzer as shown below.



2. Click on the button as shown below on the DeXipher homepage.

3. Select the well for detection and import the needed information of each detection samples. Click next step as figure shown as below.

4. Enter the “Assay Name” for the assay and click icon as shown below for image pattern decoding and fluorescent signal acquisition.

5. Export the results for data analysis or calculation or the pdf report.

11. DISCLAIMERS

Negative test result

A negative test result means the IntelliPlex BRAF V600 Mutation Kit test did not detect the nucleotide changes on codon 600 of the BRAF gene. It does not preclude the nucleotide changes on codon 600 of the BARF gene. Moreover, false negative test results may be due to experimental errors or other causes. Clinical interpretation of the results should be made in combination with all other clinical findings.

Positive test result

A positive test result means that the IntelliPlex BRAF V600 Mutation Kit detected the nucleotide changes on codon 600 of the BARF gene. It does not preclude the possibility that the specimen did not have mutation on codon 600 of the BRAF gene. False positive test results may be caused by experimental errors or other causes. Clinical interpretation of the results should be made in combination with all other clinical findings.

12. INTERPRETATION OF RESULTS

Table 4: Interpretation of Result

Test Result	Report Result	Interpretation
Mutation Detected	V600E1, V600E2, V600D, V600G, V600K, V600R, V600M	Mutation detected on the targeted BRAF regions
Mutation Not Detected	None	Mutation not detected on the targeted BRAF regions
Invalid Assay	Invalid	**Possible Cause:** 1. PCR Inhibition 2. SAPE Reaction Failed 3. Low Input or Low Quality of Sample DNA 4. Low πCode MicroDisc Count 5. No πCode MicroDisc Detected 6. Blank πCode MicroDisc Control Failed

13. ANALYTICAL PERFORMANCE

Limit of Blank (LoB)

The limit of blank (LoB) values were determined by 8 replicates of wild-type BRAF cell line (K562) across 3 days and duplicates of 10 wild-type BRAF FFPE specimens across 3 days. Based on the results, the maximum analytical signal intensity values for each mutation were used as the cutoff values for each targeted mutation of the assays.

The 10 FFPE specimens were obtained from Zytogenex, under IRB approval from MacKay Memorial Hospital and Health GeneTech, respectively. Each FFPE specimens were confirmed as BRAF wild-type specimens by Sanger sequencing or Pyrosequencing before LoB determination tests.

"Mutation Not Detected" results were only observed in the samples presence of BRAF wild type DNA.

Limit of Detection (LoD)

The limit of detection (LoD) of IntelliPlex BRAF V600 Mutation Kit was determined for 7 nucleotide changes on exon 15 of the BRAF gene. Each mutant DNA was tested from 5 different mutation levels of plasmid blended with wild-type BRAF cell line DNA (K562). The mutation level range are serial diluted from 2.5% to 0.05% mutation level (2.5%, 1%, 0.5%, 0.25%, 0.1%, and 0.05%) and DNA from BRAF wild-type FFPE as 0%. The LoD was performed by using 2 lots of IntelliPlex BRAF V600 Mutation Kit to test various mutation level for each mutation site, respectively. Each level of DNA was tested with 7 replicates across 3 days per reagent lot. The LoDs of each lot were determined based on a positive hit rate at 95% in PriProbit analysis as shown in table 5.

Table 5. Limit of Detection (LoD) of each BRAF Mutation

Mutation	Lot4	Lot5	Final LoD
V600E1	1.30%	1.10%	1.30%
V600E2	0.64%	0.94%	0.94%
V600D	0.74%	1.57%	1.57%
V600G	0.29%	0.11%	0.29%
V600K	0.42%	0.26%	0.42%
V600R	0.49%	0.42%	0.49%
V600M	1.22%	1.06%	1.22%

Repeatability

The repeatability of IntelliPlex BRAF V600 Mutation Kit was evaluated across three reagent lots, 2 operators, 2 sets of instrument and 10 non-consecutive testing days. Four replicate runs were performed per reagent lot per day for a total of 40 runs at one site. Repeatability of IntelliPlex BRAF V600 Mutation Kit was demonstrated for four wild-type FFPE samples, low level mutant (1x-3x LoD) and high level mutant (2x-9x LoD). The accuracy of the all testing level was at least 98% (39/40) across all variance combined.

Table 6. Repeatability Accuracy of Each BRAF Mutation

Mutation	Mutation Level (%)	Mutation Detected	Mutation Not Detected	Accuracy (%)
V600E1	8.47	40	0	100
	2.82	40	0	100
V600E2	6.42	40	0	100
	2.14	40	0	100
V600D	1.85	40	0	100
V600G	2.50	40	0	100
	0.83	40	0	100
V600K	1.01	40	0	100
	0.34	39	1	98
V600R	2.73	40	0	100
	0.91	40	0	100
V600M	6.35	40	0	100
	2.12	40	0	100
WT FFPE 1	-	0	20	100
WT FFPE 2	-	0	20	100
WT FFPE 3	-	0	20	100
WT FFPE 4	-	0	20	100

Reproducibility

The reproducibility of the IntelliPlex BRAF V600 Mutation Kit was examined using the same panel of DNA and wild-type sample blend used in repeatability tests. This study was tested across three testing sites with two operators per site, one reagent lot, one set of instrument per site and 5 non-consecutive days. Each run included two replicates of each mutation level and four wild type samples. Total of 60 valid runs for each mutations and

120 valid runs for wild type samples were completed across three sites in 5 days of testing. All the accuracy were at least 95% (57/60) (table 7). Across all variance components (i.e., site/instrument, operator, and day), the overall coefficient of variation is smaller than 5% across all panel members (table 8).

Table 7. Reproducibility Accuracy of Each BRAF Mutation

Mutation	Mutation Level (%)	Mutation Detected	Mutation Not Detected	Accuracy (%)
V600E1	8.47	60	0	100
	2.82	59	1	98.3
V600E2	6.42	60	0	100
	2.14	60	0	100
V600D	1.85	60	0	100
V600G	2.50	60	0	100
	0.83	60	0	100
V600K	1.01	60	0	100
	0.34	57	3	95
V600R	2.73	60	0	100
	0.91	60	0	100
V600M	6.35	60	0	100
	2.12	60	0	100

Table 8. Reproducibility Coefficient of Each BRAF Mutation

Mutation	Mutation Level (%)	Overall Coefficient
V600E1	8.47	0.00%
	2.82	2.94%
V600E2	6.42	0.00%
	2.14	0.00%
V600D	1.85	0.00%
V600G	2.5	0.00%
	0.83	0.00%
V600K	1.01	0.00%
	0.34	5.26%
V600R	2.73	0.00%
	0.91	0.00%
V600M	6.35	0.00%
	2.12	0.00%

Cross-Reactivity

The cross-reactivity was evaluated by testing the BRAF homolog plasmids (ARAF and RAF1) to eliminate the false positive results. The tested plasmids were divided into two groups: one was DNA blended with 5% of BRAF V600E1 in a background of ARAF and RAF1 DNA. Another one was tested with 100% of ARAF and RAF1 DNA. Each group was operated with triplicates per day in 2 days testing. The results demonstrated that there was no cross reactivity with any of the tested samples (table 9).

Table 9. Cross Reactivity Tests of BRAF between ARAF and RAF1 DNA

Test Group	Total Tests	Test Results	Accuracy %
ARAF A- 95% V600E1- 5%	6	V600E1	100%
ARAF A- 100%	6	WT	100%
ARAF B- 95% V600E1- 5%	6	V600E1	100%
ARAF B- 100%	6	WT	100%
ARAF 13- 95% V600E1- 5%	6	V600E1	100%
ARAF 13- 100%	6	WT	100%
RAF1 10- 95% V600E1- 5%	6	V600E1	100%
RAF1 10- 100%	6	WT	100%
RAF1 14- 95% V600E1- 5%	6	V600E1	100%
RAF1 14- 100%	6	WT	100%

Cross-Contamination

The test is designed to access the cross-contamination during all assay operation steps which may lead to the false positive results. The tested wild-type FFPE samples and BRAF V600E1 mutation FFPE samples were placed in rotated order between wells in the 96-well plate. The results demonstrated that there was no cross-contamination during all assay operation steps.

Interference

The test is designed to evaluate the impact of potentially carrying over substances from QIAamp DNA FFPE Tissue Kit. The BRAF V600E1 mutation FFPE samples and each potential interference substances (Listed as table 10) were tested in three replicates. The result indicated the interference substances will not interfere the performance of the Intelliplex BRAF V600 Mutation Kit.

Table 10. The tested interfering substances

Interfering Substance	Assumed Interference Residue Volume (ul / 20ul DNA)
Xylene	$4*10^{-5}$
Ethanol	$2.7*10^{-4}$
Buffer ATL	$1.08*10^{-4}$
Proteinase K	$2.64*10^{-6}$
Buffer AL	$2.66*10^{-4}$
Wash Buffer AW1	0.1
Wash Buffer AW2	1

14. SYMBOLS

Symbol	Explanation	Symbol	Explanation
IVD	In-vitro diagnostic use	REF	Catalog number
LOT	Batch number		Consult instructions for use
	Manufacturer		Use by Date
	Temperature limitation		Caution
Σ	Contains sufficient for <n> tests		Date of Manufacture
CE	European Union Conformity	EC REP	European Authorized Representative

15. REFERENCES

1. Di Nicolantonio F, Martini M, Molinari F, Sartore-Bianchi A, Arena S, Saletti P, De Dosso S, Mazzucchelli L, Frattini M, Siena S, Bardelli A. (2008) Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J. Clin. Oncol. 2008; 26:5705–5712.
2. Tol J, Nagtegaal ID, Punt CJ. (2009) BRAF mutation in metastatic colorectal cancer. N. Engl. J. Med. 2009;361:98–99.
3. Ogino S, Nosho K, Kirkner GJ, Kawasaki T, Meyerhardt JA, Loda M, Giovannucci EL, Fuchs CS. (2009) CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut. 2009;58:90-96.
4. Namba H, Nakashima M, Hayashi T, et al. (2003) Clinical implication of hot spot BRAF mutation, V599E, in papillary thyroid cancers. J Clin Endocrinol Metab 2003;88:4393–7.
5. Rowe LR, Bentz BG, Bentz JS. (2006) Utility of BRAF V600E mutation detection in cytologically indeterminate thyroid nodules. Cytojournal 2006;3(1):10.
6. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Neil Davis, Ed Dicks, Rebecca Ewing, Yvonne Floyd, Kristian Gray, Sarah Hall, Rachel Hawes, Jaime Hughes, Vivian Kosmidou, Andrew Menzies, Catherine Mould, Adrian Parker, Claire Stevens, Stephen Watt, Steven Hooper, Rebecca Wilson, Hiran Jayatilake, Gusterson Barry A, Colin Cooper, Janet Shipley, Darren Hargrave, Katherine Pritchard-Jones, Norman Maitland, Georgia Chenevix-Trench, Riggins Gregory J, Bigner Darell D, Giuseppe Palmieri, Antonio Cossu, Adrienne Flanagan, Andrew Nicholson, Ho Judy WC, Leung Suet Y, Yuen Siu T, Weber Barbara L, Seigler Hilliard F, Darrow Timothy L, Hugh Paterson, Richard Marais, Marshall Christopher J, Richard Wooster, Richard Wooster, Michael R, Stratton P, Andrew Futreal. (2002) Mutations of the BRAF gene in human cancer. Nature 2002, 417:949–954.
7. Bollag G, Hirth P, Tsai J, Zhang J, Ibrahim PN, Cho H, Spevak W, Zhang C, Zhang Y, Habets G, Burton Elizabeth A, Bernice Wong, Garson Tsang, Powell B, West Brian L, Shellooe R, Marimuthu A, Nguyen H, Zhang KYJ, Artis DR, Schlessinger J, Su F, Higgins B, Iyer R, D'Andrea K, Koehler A, Stumm M, Lin PS, Lee RJ, Grippo J, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, Chapman PB, Flaherty KT, Xu X, Nathanson KL, Nolop K. (2010) Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. Nature 2010, 467:596–599.
8. Sosman JA, Kim KB, Schuchter L, Gonzalez R, Pavlick AC, Weber JS, McArthur GA, Hutson TE, Moschos SJ, Flaherty KT, Hersey P, Kefford R, Lawrence D, MD, Puzanov I, Lewis KD, Amaravadi RK, Chmielowski B, Lawrence HJ, ShyrY, Ye F, Li J, Nolop KB, Lee RJ, Joe

AK, Ribas. (2012) Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. N Engl J Med 2012, 366:707–714.
9. Rubinstein JC, Sznol M, Pavlick AC, Ariyan S, Cheng E, Bacchiocchi A, Kluger HM, Narayan D, Halaban R. (2010) Incidence of the V600K mutation among melanoma patients with BRAF mutations, and potential therapeutic response to the specific BRAF inhibitor PLX4032. J Transl Med 2010, 8:67.
10. Hoeflich KP, Gray DC, Eby MT, Tien JY, Wong L, Bower Jet al. (2006). Oncogenic BRAF is required for tumor growth and maintenance in melanoma models. Cancer Res 66: 999–1006.
11. Vemurafenib in Multiple Nonmelanoma Cancers with BRAF V600 Mutations. N Engl J Med 2015; 373:726-736August 20, 2015

Notice to User

The use of this product and the associated PlexBio instrumentation is covered by one or more issued (US 9,063,044B2) and pending US and foreign patents owned by PlexBio Co., Ltd. The purchase of this product includes nontransferable rights to use this amount of the product to practice the methods described therein. No general patent or other license of any kind other than this specific right of use from purchase is granted. For further information on purchasing licenses for other applications can be obtained from PlexBio Co., Ltd. 6F-1, No. 351, Yangguang St., Neihu District, Taipei City 11491, Taiwan. The LNA technology in this product is provided under an agreement between Exiqon A/S and PlexBio Co., Ltd., and is covered by patents/patent applications owned by Exiqon A/S.

Trademarks

PLEXBIO , πCode™ ,DeXipher™, IntelliPlex™, DigiPlex™, πCode™ , PlexBio™, are trademarks or registered trademarks of PlexBio Co., Ltd. All other product names and any registered and unregistered trademarks mentioned are used for identification purposes only and remain the exclusive property of their respective owners.

Copyright

©2017 PlexBio Co., Ltd. All rights reserved.