IntelliPlex™ Lung Cancer Panel – DNA

REF **82032** **24 Reactions**

RUO **For Research Use Only**

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IMPORTANT:
Read the instructions carefully prior to use

1. INTENDED USE

The **IntelliPlex Lung Cancer Panel – DNA**, based on πCode™ technology and PlexBio's instrument platform, is an in vitro molecular assay intended for the qualitative identification of 74 DNA mutations in the KRAS, NRAS, PIK3CA, BRAF, EGFR, HER2, MEK1 and AKT1 genes using DNA derived from formalin-fixed paraffin-embedded (FFPE) of patients with non-small cell lung cancer (NSCLC). The kit is to be used by trained personnel in a professional laboratory environment. This product is intended for research use only, and not for use in clinical diagnostic or treatment procedures.

2. INTRODUCTION

Studies of NSCLC has identified recurrent 'driver' mutations that occur in multiple oncogenes, including AKT1, ALK, BRAF, EGFR, HER2, KRAS, MEK1, MET, NRAS, PIK3CA, RET, and ROS1, and these markers serve as the basis for the molecular classification of NSCLC. Both DNA mutations and gene variants contribute to oncogenesis in NSCLC.

SelectAmp and πCode technology enables the multiplex, high sensitivity, single-well detection of mutations from specimens containing large amounts of wild-type genomic DNA with significantly reduced sample requirement compared to conventional methods. The **IntelliPlex Lung Cancer Panel – DNA** identifies 74 DNA mutations (Table 1).

Table 1. Mutations Detected

Gene	Exon	Amino Acid Change	Nucleotide change	COSMIC ID
KRAS	Exon 2	p.G12S	c.34G>A (GGT>AGT)	517
		p.G12R	c.34G>C (GGT>CGT)	518
		p.G12C	c.34G>T (GGT>TGT)	516
		p.G12D	c.35G>A (GGT>GAT)	521
		p.G12A	c.35G>C (GGT>GCT)	522
		p.G12V	c.35G>T (GGT>GTT)	520
		p.G13D	c.38G>A (GGC>GAC)	532
		p.G13C	c.37G>T (GGC>TGC)	527
	Exon 3	p.Q61H	c.183A>C (CAA>CAC)	554

Gene	Exon	Amino Acid Change	Nucleotide change	COSMIC ID
		p.Q61H	c.183A>T (CAA>CAT)	555
		p.Q61L	c.182A>T (CAA>CTA)	553
		p.Q61K	c.181C>A (CAA>AAA)	549
		p.Q61R	c.182A>G (CAA>CGA)	552
NRAS	Exon 3	p.Q61K	c.181C>A (CAA>AAA)	580
		p.Q61R	c.182A>G (CAA>CGA)	584
		p.Q61L	c.182A>T (CAA>CTA)	583
		p.Q61H	c.183A>C (CAA>CAC)	586
		p.Q61H	c.183A>T (CAA>CAT)	585
PIK3CA	Exon 9	p.E542K	c.1624G>A	760
		p.E545K	c.1633G>A	763
		p.E545Q	c.1633G>C	27133
	Exon 20	p.H1047R	c.3140A>G	775
		p.H1047L	c.3140A>T	776
BRAF	Exon 15	p.V600E1	c.1799T>A	476
EGFR	Exon 18	p.G719A	c.2156G>C	6239
		p.G719S	c.2155G>A	6252
		p.G719C	c.2155G>T	6253
	Exon 19	p.L747_T751>S	c.2240_2251del12	6210
		p.L747_E749del	c.2239_2247del9	6218
		p.E746_S752>D	c.2238_2255del18	6220
		p.E746_A750del	c.2235_2249del15	6223
		p.E746_A750del	c.2236_2250del15	6225
		p.L747_T751del	c.2239_2253del15	6254
		p.L747_T751del	c.2240_2254del15	12369
		p.L747_T751del	c.2238_2252del15	23571
		p.L747_S752del	c.2239_2256del18	6255
		p.E746_S752>A	c.2237_2254del18	12367
		p.L747_P753>S	c.2240_2257del18	12370
		p.L747_A750>P	c.2239_2248TTAAGAGAAG>C	12382
		p.L747_T751>P	c.2239_2251>C	12383
		p.E746_S752>V	c.2237_2255>T	12384
		p.E746_S752>I	c.2235_2255>AAT	12385
		p.E746_T751>V	c.2237_2252>T	12386
		p.L747_P753>Q	c.2239_2258>CA	12387
		p.L747_S752>Q	c.2239_2256>CAA	12403

Gene	Exon	Amino Acid Change	Nucleotide change	COSMIC ID
		p.E746_T751>VA	c.2237_2253>TTGCT	12416
		p.L747_T751>Q	c.2238_2252>GCA	12419
		p.L747_A750>P	c.2238_2248>GC	12422
		p.E746_T751>A	c.2237_2251del15	12678
		p.E746_T751del	c.2236_2253del18	12728
		p.E746_A750>IP	c.2235_2248>AATTC	13550
		p.E746_T751>I	c.2235_2252>AAT	13551
		p.E746_T751>IP	c.2235_2251>AATTC	13552
		p.S752_I759delSPKANKEI	c.2254_2277del24	6256
		p.S752_I759delSPKANKEI	c.2253_2276del24	13556
		p.E746_P753>VS	c.2237_2257>TCT	18427
		p.K745_E749del	c.2233_2247del15	26038
	Exon 20	p.T790M	c.2369C>T	6240
		p.C797S	c.2389T>A	6493937
		p.C797S	c.2390G>C	5945664
		p.S768I	c.2303G>T	6241
		p.V769_D770insASV	c.2307_2308ins9GCCAGCGTG	12376
		p.H773_V774insH	c.2319_2320insCAC	12377
		p.D770_N771insG	c.2310_2311insGGT	12378
		p.D770_N771insSVD	c.2311_2312ins9GCGTGGACA	13428
		p.V769_D770insASV	c.2309_2310AC>CCAGCGTGGAT	13558
	Exon 21	p.L858R	c.2573T>G	6224
		p.L858R	c.2573_2574TG>GT	12429
		p.L861Q	c.2582T>A	6213
AKT1	Exon 4	p.E17K	c.49G>A	33765
MEK1	Exon 2	p.Q56P	c.167A>C	1235481
		p.K57N	c.171G>T	1235478
HER2	Exon 20	p.Ala775_Gly776insYVMA	c.2324_2325ins12	20959
		p.Ala775_Gly776insYVMA	c.2325_2326ins12	12558

3. TECHNOLOGICAL PRINCIPLES

πCode MicroDisc

πCode MicroDisc is manufactured to generate more than 85,000 distinct circular image patterns for multiplexing applications. Each πCode MicroDisc has a distinct circular image pattern, which corresponds to a specific capture agent conjugated to the surface of the disc. πCode tagged with different capture agents are pooled, enabling specific detection of multiple analytes in a one well reaction.

Detection Principle

The test is based on five processes:

1. DNA extraction from formalin-fixed paraffin-embedded (FFPE) specimens
2. Mutation -specific multiplex PCR amplification
3. Hybridization of PCR amplicons with mutation/ variants-specific probe tagged πCode in a one well reaction
4. Fluorescent labeling with streptavidin-phycoerythrin
5. Image pattern decoding and fluorescent signal detection by the PlexBio™ 100 Fluorescent Analyzer

4. WARNINGS AND PRECAUTIONS

- For research use only. Not for use in diagnostic procedures.
- This assay kit should only be used by qualified laboratory personnel.
- Separate, dedicated rooms and equipment for pre- and post- PCR process with unidirectional manner to avoid any contaminations are required.
- Pre-PCR process preparation should be operated in laminar flow hood to avoid contamination.
- Do not use a kit or reagent past its expiration date.
- Reagent components have been diluted optimally. Further dilution of the component reagents is not recommended.
- Specimens should be handled as infectious material. Please follow universal precautions 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 may 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.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
- Material Safety Data Sheets (SDS) are available upon request from PlexBio Customer Service.

5. QUALITY CONTROL

The **IntelliPlex Lung Cancer Panel – DNA** contains a series of internal control πCode MicroDiscs that monitor the PCR amplification, SA-PE incubation procedure and background noise. Those controls must meet the specification in each test well with intensities above the cutoffs from the same run, or the run will be considered failed. The external controls (positive control and negative control) monitor the whole testing procedure to prevent false positive and false negative results. The test is considered invalid if any of the controls fail.

6. KIT COMPONENTS

The **IntelliPlex Lung Cancer Panel – DNA** contains sufficient reagents for up to 24 tests. Kit components include:

(1) cLCP - DNA Reaction Mix
Ref. No.: 20484-R
Quantity & Volume: 1 vial, 240 µL/vial
Description: For PCR amplification
Contents: MyFi 5X Reaction Buffer, MyFi DNA polymerase (Microbial), buffered solution containing $MgSO_4$ and dNTPs

(2) cLCP - DNA Primer Mix
Ref. No.: 20485-R
Quantity & Volume: 1 vial, 240 µL/vial
Description: For PCR amplification
Contents: ~4 µM Primer (including biotin-labeled primers)

(3) cLCP – FFPE DNA πCode MicroDisc
Ref. No.: 20689-R
Quantity & Volume: 1 vial, 480 µL/vial
Description: For PCR amplicon capture
Contents: Glycerol, πCode, Phosphate buffered saline, 0.1% Albumin from bovine (Biological), <0.1% EDTA, <0.1% Sodium azide

(4) cLCP - DNA POS Control
Ref. No.: 20487-R
Quantity & Volume: 1 vial, 120 µL/vial
Description: Assay positive control
Contents: Cell line DNA, Tris-EDTA Buffer

(5) NEG Control
Ref. No.: 20549-R
Quantity & Volume: 1 vial, 120 µL/vial
Description: Assay negative control
Contents: Nuclease-free water

(6) cLCP - DNA Hy Buffer
Ref. No.: 20489-R
Quantity & Volume: 1 bottle, 2.4 mL/bottle
Description: For hybridization
Contents: Saline-Sodium Phosphate-EDTA

(7) SA-PE Solution
Ref. No.: 20302
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 (Biological), <0.1% Sodium azide

7. MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

Required products for compatibility with IntelliPlex kits:

- 96-well plate (PlexBio; Cat. No. 80025 or Greiner Bio-one; Cat. No. 655101)
- IntelliPlex™ 1000 πCode Processor (PlexBio; Cat. No. 80033)
- PlexBio™ 100 Fluorescent Analyzer (PlexBio; Cat. No. 80000)

- U Tray (PlexBio; Cat. No. 80023)
- V Tray (PlexBio; Cat. No. 80024)
- DeXipher™ RUO (Required: PlexBio; Cat. No. 80050)
- 10X Assay Wash Buffer (PlexBio; Cat. No. 80220)
- Deionized water for dilution of 10X Assay Wash Buffer

Required components:

- (Recommended) FFPE DNA extraction kit (QIAamp DNA FFPE Tissue Kit, Qiagen; Cat. No. 56404 or equivalent)
- Qubit™ Fluorometer with dedicated quantitative reagents (Invitrogen; any models) or equivalent
- Clean tubes for PCR reaction (Gunster; Cat. No. MB-P08A or equivalent)
- Dedicated micropipette
- Filter tips for micropipette
- Disposable powderless gloves
- Vortex mixer
- Micro-centrifuge
- Thermocycler (Recommended: MiniAmp™ Thermal Cycler, Applied Biosystems™; Cat. No. A37834 or equivalent)
- Computer (Recommended: PlexBio; Cat. No. 80002)

8. STORAGE, STABILITY AND TRANSPORTATION

Storage

The kit components of the **IntelliPlex Lung Cancer Panel – DNA** should be stored at 2°C to 8°C.

Stability

Do not use any kit that has expired. All unopened 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 kit is 2-8°C. If the kit package or components is incomplete, please contact PlexBio customer service (service@plexbio.com).

9. INSTRUMENTS AND SOFTWARE

Instruments

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

Software Installation

The **IntelliPlex Lung Cancer Panel – DNA** has a designated Kit App and ENC file. The Kit App contains the πCode target assignments and the ENC file includes the lot number and expiration date. Please make sure you have the Kit App installed and the ENC file imported into DeXipher before your first assay run.

Kit App Installation

1. Visit www.plexbio.com and download the **LCP-FFPE DNA_RNA App**.
2. Click on the “Installer” in the APP folder and follow the instructions to complete Kit App installation.

NOTE: The Kit App only needs to be installed once. Version updates will be notified by customer service.

ENC File Installation

1. Visit www.plexbio.com and download the **LCP-FFPE DNA_RNA** ENC file. Each kit lot number will have a unique ENC file, so you will need to download a new ENC file each time you purchase a kit with a different lot number. Make sure to select the ENC file with the lot number that corresponds to your kit.
2. Save the ENC file to your computer.
3. Follow the PlexBio 100 Fluorescent Analyzer User Manual to import the ENC.

10. SPECIMENS

Specimen Collection

The **IntelliPlex Lung Cancer Panel – DNA** has been validated to be used for formalin-fixed paraffin embedded tissues (FFPET) from non-small cell lung cancer (NSCLC) tumor tissues. It is recommended to extract FFPE DNA with QIAamp DNA FFPE Tissue Kit (Cat. No. 56404) for downstream PCR amplification.

NOTE FOR USE OF FFPET:

- FFPET specimens may be transported and stored at room temperature for up to 12 months after the date of tissue collection and processing. The optimal tissue fixation time for test should be less than 72 hr.
- Only FFPET sections of 10-µm thickness containing at least 10% tumor content are to be used in the KRAS Mutation Test. Any specimen containing less than 10% tumor content should be macro-dissected prior to deparaffinization.
- Do not use stained FFPE specimens which could generate invalid and/or incorrect results.

Storage of Extracted DNA

Extracted DNA can be stored at 2°C to 8°C for immediate use (≤ 24 hours), or at -15°C to -25°C for long-term (> 24 hours) storage. Do not subject the extracted DNA to repeated freeze/thaw cycles.

11. BEFORE YOU START

1. Check that the Kit App has been installed and the lot specific ENC file has been imported to DeXipher.
2. It is recommended to use QIAamp DNA FFPE Tissue Kit to extract FFPE DNA, please follow the extraction kit instructions and ensure that you have 20 µL of extracted DNA (0.5 ng/µL) ready for analysis.

12. ASSAY PROCEDURE

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

12.1 DNA Extraction, Quantification and PCR Setup

1. Follow the QIAamp DNA FFPE Tissue Kit instructions to purify and store the extracted samples properly.
2. Quantify the extracted DNA using a Qubit Fluorometer (or equivalent) according to the manufacturer's protocol.
3. The extracted sample must be > 0.5 ng/µL to perform the **IntelliPlex Lung Cancer Panel – DNA**. Each PCR reaction uses 20 µL of a 0.5 ng/µL DNA working stock (10 ng DNA input). Please prepare enough working stock for samples before preparing PCR reactions.
4. Vortex to mix each sample before use.
5. Spin down and keep samples on ice.

6. Prepare the PCR Reaction as follows and be aware of cross-contamination during processing sample DNA:

For each PCR reaction:

cLCP-DNA Reaction Mix	10 µL
cLCP-DNA Primer Mix	10 µL
Sample/ POS Control/NEG Control	20 µL
Total volume	40 µL

NOTE:

- The amount of Reaction Mix and Primer Mix required for a Master Mix depends on the number of reactions. Always prepare a surplus.
- Both POS Control and NEG Control are required for test validity and report generation and must be included in each assay run.

12.2 Multiplex PCR Amplification

1. Mix by tapping the PCR Reaction tubes and spin down before placing the tubes in the thermocycler. Set up the PCR program conditions as below:

PCR Program Conditions:

Temp. (˚C)	Time	Cycles
95	5 min	-
95	20 sec	9
70	20 sec	
60~52*	20 sec	
60	25 sec	
95	20 sec	27
70	20 sec	
55	20 sec	
60	25 sec	
4	Hold	-

***NOTE:**

- *Ramp rate: 3°C/sec (ABI MiniAmp™; Cat. No. A37834).*
- *Temp. Setting for 60~52 indicates decreasing 1°C per cycle.*

12.3 DNA Hybridization and SA-PE Reaction

1. **Prepare 1X Assay Wash Buffer:** Transfer 100mL of the 10X Assay Wash Buffer (PlexBio; Ref: 80220) to the IntelliPlex 1000 πCode Processor 1L Wash Buffer bottle and add 900 ml deionized water. Mix by swirling.

NOTE: The prepared 1X Assay Wash Buffer can be used for up to one week. Please always check the Wash Buffer is sufficient for assay runs. Additional 10X Assay Wash Buffer can be ordered from PlexBio (Ref. No: 80220).

IntelliPlex 1000 πCode Processor Assay Wash Buffer consumption:

Procedure	Assay Wash Buffer Consumption (mL)
Self-test	50
DNA & RNA program (1 lane, up to 8 tests)	150
DNA & RNA program (3 lanes, up to 24 tests)	220

2. **Add 20 µL cLCP – FFPE DNA πCode MicroDisc to 96 well plate:** Mix by vortexing the πCode for 10 seconds, then, by pipetting, add 20 µL of the πCode to each well directly. Vortex the tube of πCode every four wells in between dispensing to ensure homogeneous suspension.

   NOTE: Each amplified PCR products (including samples, POS and NEG control) should be added into wells lane wise, in order of A1, B1…H1 and followed by A2, B2…H2 and so on.

3. **Add 100 µL of cLCP - DNA Hy Buffer** to each well.

4. Spin down the PCR products.

5. **Denature the PCR products** on the thermocycler by heating at 95˚C for **7** minutes, immediately cooled on ice/cooler or thermocycler to ensure the denatured status. Spin down before use. Use immediately (within 1 hour after denaturation).

   NOTE: Pay attention to the lid temperature of thermocycler while taking out the denatured PCR products.

6. **Add 20 µL of the denatured PCR products** to each well.

7. **Pipet the desired volume of SA-PE solution** into the V Tray in SA-PE solution tank. Please note that the dead volume of V Tray is **500 µL** for up to 6 selected lanes or **800 µL** if more than 6 lanes are selected. The minimum usage of SA-PE is **one lane (900 µL)**.

   Calculation Example:
   For a 3-lane reaction, the required SA-PE solution volume is at least:
   400 µL x 3 lanes + 500 µL(dead volume)= 1.7 mL
   It is recommended to add extra solution volume into the V Tray to ensure sufficient dispensing volume.

   NOTE: Required SA-PE Solution by Lane(s):

Number of Processed Lane(s)	Required SA-PE Solution (µL)
1	900
2	1300
3	1700
4	2100
5	2500
6	2900
7	3600
8	4000
9	4400
10	4800
11	5200
12	5600

   - SA-PE solution should be kept in the dark.
   - **Do not** reuse the leftover SA-PE solution and V Tray tank. Replace a new V Tray with every assay run.

8. **Run hybridization and wash:** This assay uses the **DNA/RNA program** in the **Molecular Assay** window of the IntelliPlex 1000 πCode Processor. Refer to the IntelliPlex 1000 πCode Processor operation manual and follow the instructions to run the built-in assay program (Homepage/ Molecular Assay/ Well Selection/ DNA/RNA/ Confirm procedure conditions/ Start Running). The plate will be ready for decoding once the program is finished.

   NOTE:

   - IntelliPlex 1000 πCode Processor must be maintained properly and regularly.
   - **Do not** open the door when the instrument is in operation.

- The kit contains sufficient reagents for 6 runs of 4 samples (including POS and NEG controls) for a maximum of 24 tests.

12.4 Image Decoding and Fluorescent Detection

1. Follow the PlexBio 100 Fluorescent Analyzer User Manual to set up the read.

   NOTE:

   - PlexBio 100 Fluorescent Analyzer must be calibrated regularly (once per month).
   - Check that the correct ENC file has been imported.
2. Launch DeXipher to run **Qualitative Assay**.
3. Mark the wells for sample, positive and negative controls.
4. Enter Assay name and place the plate into the device with the correct orientation as shown on the screen.
5. The raw data will be analyzed through the kit ENC to generate the mutation call report.

NOTE:
A single run can include from 2 to 96 tests (including POS and NEG controls) per 96 well Microwell plate. When running more than 24 specimens, multiple **IntelliPlex Lung Cancer Panel – DNA** of the same lot will be required.

13. DISCLAIMERS

Negative Test Result

A negative test result means that the targeted mutation was not detected by the kit. It does not preclude a positive result of the targeted mutation. Experimental errors or other causes may lead to false negative results. Interpretation of the results should consider these possibilities.

Positive Test Result

A positive test result means that the targeted mutation was detected by the kit. It does not preclude a negative result for the targeted mutation. Experimental errors or other causes may lead to false positive results. Interpretation of the results should consider these possibilities.

14. INTERPRETATION OF RESULT

The report generated by DeXipher includes the results of controls and samples tested in the same run. The result of external controls (POS Control and NEG Control) must be “Pass”. Otherwise, failed POS or NEG Control renders the whole assay run invalid, and the result of tested samples will not be reported.
If the result POS Control and NEG Control are “Pass”, the result of each tested sample will be reported on separate sheets in detail. For each tested sample, its internal controls (Reference Gene Control, Internal Control, SA-PE Monitor Control, πCode MicroDiscs Count, πCode MicroDiscs Combination and Blank Control) must be “Pass”, or the test of that sample is invalid. The detection result of invalid samples will not be shown. However, failed Reference Gene Control and Internal Control do not negate samples with mutation detected. A positive sample with failed Reference Gene Control is considered valid. The detection result of target genes will be shown for each valid sample.

Refer to the chapter **“Troubleshooting”** for control failure issues.

Table 2. Interpretation of Results

Test Result	Explanation	Action
Mutation Detected	Refer to Table 1	Targeted mutation detected
Mutation Not Detected	None	Targeted mutation not detected

Test Result	Explanation	Action
Result Not Shown	The test is **INVALID** because external controls failed OR at least one of internal controls failed	See the chapter **"Troubleshooting"** for instructions and retest.

NOTE:

- All runs and specimen validation were performed by the dedicated KIT APP along with IntelliPlex 1000 πCode Processor and PlexBio 100 Fluorescent Analyzer.
- "Mutation Detected" indicates that the signal for at least one mutation site is greater than the cutoff value of the corresponding target.

15. ANALYTICAL PERFORMANCE

Limit of Blank (LoB)

The limit of blank (LoB) values were determined by 2 operators performing four replicates of 37 wild-type FFPE specimens across three days on 2 reagent lots and 2 sets of instruments. Origins of the FFPE specimens include biobanks from France, US, and Taiwan.

The cut-off values for each mutation site were then determined by 1.4-folds of the measured maximum fluorescent signal intensity values from the LoB study.

Only "**Mutation Not Detected**" results were observed in these wild type specimens.

Limit of Detection (LoD)

The limit of detection (LoD) was determined using a dilution series (ranging from 0.1~5%) containing different levels of mutant plasmid DNA blended in a background of wild type FFPE DNA. Each dilution was tested with 21 replicates across 3 days per reagent lot across three operators and two reagent lots. The LoDs were determined based on a positive hit rate at 95% in PriProbit analysis (Table 3). The LoDs ranged from 0.1~7.5%.

Table 3. Limit of Detection (LoD)

Gene	Amino Acid Change	Nucleotide Change	LoD (% Mutation)
KRAS	p.G12S	c.34G>A (GGT>AGT)	0.10%
	p.G12R	c.34G>C (GGT>CGT)	0.24%
	p.G12C	c.34G>T (GGT>TGT)	0.62%
	p.G12D	c.35G>A (GGT>GAT)	0.68%
	p.G12A	c.35G>C (GGT>GCT)	0.64%
	p.G12V	c.35G>T (GGT>GTT)	0.32%
	p.G13D	c.38G>A (GGC>GAC)	0.10%
	p.G13C	c.37G>T (GGC>TGC)	0.58%
	p.Q61H	c.183A>C (CAA>CAC)	0.33%
	p.Q61H	c.183A>T (CAA>CAT)	0.23%
	p.Q61L	c.182A>T (CAA>CTA)	0.79%
	p.Q61K	c.181C>A (CAA>AAA)	0.56%
	p.Q61R	c.182A>G (CAA>CGA)	1.07%
NRAS	p.Q61K	c.181C>A (CAA>AAA)	0.45%
	p.Q61R	c.182A>G (CAA>CGA)	1.58%
	p.Q61L	c.182A>T (CAA>CTA)	2.46%

Gene	Amino Acid Change	Nucleotide Change	LoD (% Mutation)
	p.Q61H	c.183A>C (CAA>CAC)	0.78%
	p.Q61H	c.183A>T (CAA>CAT)	0.99%
PIK3CA	p.E542K	c.1624G>A	1.08%
	p.E545K	c.1633G>A	0.64%
	p.E545Q	c.1633G>C	0.12%
	p.H1047R	c.3140A>G	0.11%
	p.H1047L	c.3140A>T	0.11%
BRAF	p.V600E1	c.1799T>A	0.10%
EGFR	p.G719A	c.2156G>C	0.50%
	p.G719S	c.2155G>A	1.36%
	p.G719C	c.2155G>T	0.32%
	p.L747_T751>S	c.2240_2251del12	1.00%
	p.L747_E749del	c.2239_2247del9	1.16%
	p.E746_S752>D	c.2238_2255del18	2.50%
	p.E746_A750del	c.2235_2249del15	0.55%
	p.E746_A750del	c.2236_2250del15	0.38%
	p.L747_T751del	c.2239_2253del15	7.50%
	p.L747_T751del	c.2240_2254del15	
	p.L747_T751del	c.2238_2252del15	
	p.L747_S752del	c.2239_2256del18	1.22%
	p.E746_S752>A	c.2237_2254del18	0.99%
	p.L747_P753>S	c.2240_2257del18	0.55%
	p.L747_A750>P	c.2239_2248TTAAGAGAAG>C	0.63%
	p.L747_T751>P	c.2239_2251>C	0.70%
	p.E746_S752>V	c.2237_2255>T	0.54%
	p.E746_S752>I	c.2235_2255>AAT	0.45%
	p.E746_T751>V	c.2237_2252>T	0.62%
	p.L747_P753>Q	c.2239_2258>CA	0.57%
	p.L747_S752>Q	c.2239_2256>CAA	0.24%
	p.E746_T751>VA	c.2237_2253>TTGCT	1.31%
	p.L747_T751>Q	c.2238_2252>GCA	2.50%
	p.L747_A750>P	c.2238_2248>GC	0.62%
	p.E746_T751>A	c.2237_2251del15	0.91%
	p.E746_T751del	c.2236_2253del18	0.44%
	p.E746_A750>IP	c.2235_2248>AATTC	1.00%
	p.E746_T751>I	c.2235_2252>AAT	2.50%
	p.E746_T751>IP	c.2235_2251>AATTC	1.00%

Gene	Amino Acid Change	Nucleotide Change	LoD (% Mutation)
	p.S752_I759delSPKANKEI	c.2254_2277del24	1.67%
	p.S752_I759delSPKANKEI	c.2253_2276del24	1.67%
	p.E746_P753>VS	c.2237_2257>TCT	2.50%
	p.K745_E749del	c.2233_2247del15	2.50%
	p.T790M	c.2369C>T	3.17%
	p.C797S	c.2389T>A	2.69%
	p.C797S	c.2390G>C	1.65%
	p.S768I	c.2303G>T	1.68%
	p.V769_D770insASV	c.2307_2308ins9GCCAGCGTG	7.50%
	p.H773_V774insH	c.2319_2320insCAC	0.54%
	p.D770_N771insG	c.2310_2311insGGT	0.56%
	p.D770_N771insSVD	2311_2312ins9GCGTGGACA	7.50%
	p.V769_D770insASV	2309_2310AC>CCAGCGTGGAT	7.50%
	p.L858R	c.2573T>G	0.32%
	p.L858R	c.2573_2574TG>GT	2.50%
	p.L861Q	c.2582T>A	0.12%
AKT1	p.E17K	c.49G>A	0.35%
MEK1	p.Q56P	c.167A>C	0.76%
	p.K57N	c.171G>T	0.42%
HER2	p.Ala775_Gly776insYVMA	c.2324_2325ins12	1.34%
	p.Ala775_Gly776insYVMA	c.2325_2326ins12	

16. TROUBLESHOOTING

The troubleshooting listed below addresses possible problem causes and solutions provided during assay procedures.

Problem	Possible Cause	Recommendations
No Valid Assay Assigned	1. No plate inserted. 2. Plate inserted in wrong orientation. 3. No assay APP installed. 4. No ENC file imported. 5. Two or more lots of reagent used.	1. Confirm plate is inserted and repeat reading. 2. Confirm orientation of plate and repeat reading. 3. Install assay APP and repeat reading. 4. Import ENC file and repeat reading. 5. One reagent lot used at a time.
Positive Control Fail / Negative Control Fail	1. No POS Control or NEG Control added. 2. DNase contamination. 3. Assay did not work. 4. Cross contamination between samples. 5. Wrong PC/NC wells chose.	1. Ensure POS Control and NEG Control are added. 2. Ensure all operating procedures are followed correctly. Ensure work environment is free of DNase. 3. Make sure all the assay procedures are followed correctly. 4. Clean all surfaces and equipment. Operate pre-PCR and post-PCR in the dedicated area and separate the equipment for use. 5. Choose the correct PC/NC wells and repeat reading.

Problem	Possible Cause	Recommendations
πCode MicroDiscs Count Fail	DeXipher is unable to detect sufficient πCode MicroDiscs numbers for analysis.	
	1. πCode MicroDiscs are not proper dispersed in the well.	1. Re-disperse the microplate using IntelliPlex 1000 processor, and repeat reading.
	2. Not enough πCode MicroDiscs added to well.	2. Ensure πCode MicroDiscs are well-mixed with proper amount added.
	3. Microbes exist in buffers.	3. Use freshly prepared assay wash buffer and deionized water for hybridization to reduce πCode MicroDiscs loss rate.
	4. Instruments error or malfunction.	4. Contact PlexBio Customer Service.
SA-PE Monitor Control Fail	Performance of SA-PE is assessed by the SAPE Monitor Control.	
	1. No SA-PE was added or insufficient SA-PE solution for dispensing.	1. Make sure all the assay procedures are followed correctly. Calculate sufficient SA-PE solution volume for dispensing. Repeat test.
	2. SA-PE solution inactivation.	2. Ensure correct storage condition and minimize the light exposure. Do not use SA-PE past its expiration date.
	3. Incorrect tested lanes of microplate selected for SA-PE solution dispensing.	3. Repeat assay and make sure lanes selected correctly.
Blank Control Fail	"Background" is determined by measuring MFI of an internal control that should not give a signal.	
	1. Wrong hybridization conditions.	1. Check correct hybridization program is selected
	2. Residues of SA-PE solution in wells after hybridization.	2. Ensure all buffers (Assay Wash buffer and ddH_2O) on IntelliPlex 1000 Processor are fresh-made and sufficient for washing procedures.
	3. PlexBio 100 Fluorescent Analyzer is not calibrated.	3. Perform calibration on PlexBio 100 Fluorescent Analyzer.
	4. Markings on plates.	4. Do not make any marking on plate.
Internal Control Fail	Internal Control monitors all steps in the procedure and must be positive.	
	1. PCR inhibition exists.	1. Follow instructions of sample extraction carefully. Ensure required temperature ranges and centrifugation needs are complied. Ensure complete removal of ethanol.
	2. PCR procedures are not performed correctly.	2. Make sure all PCR procedures are followed correctly. Do not to use expired materials or mixed lots of reagents. Ensure storage conditions are correct.
	3. DNase contamination.	3. Ensure all the operating procedures are followed correctly. Ensure work environment is free of DNase.
	4. Hybridization did not work.	4. Make sure all the assay procedures are followed correctly. Ensure samples are freshly heat-denatured.

Problem	Possible Cause	Recommendations
Reference Gene Fail	Reference Gene monitors quality of tested sample and must be positive.	
	1. No Sample added or absence of human-derived DNA.	1. Ensure human-derived DNA samples are added. Do not use artificial DNA as samples which may generate invalid results.
	2. Insufficient sample input for assays or poor sample quality.	2. Quantify samples and check the sample input. If still remains failed, ensure the collected samples meet specimen requirements. Retest with new samples if needed.
	3. PCR inhibition exists.	3. Follow sample extraction instructions carefully. Ensure required temperature ranges and centrifugation needs are complied. Ensure complete removal of ethanol.
	4. PCR procedures are not performed correctly.	4. Make sure all PCR procedures are followed correctly. Do not to use expired materials or mixed lots of reagents. Ensure storage conditions are correct.

17. SYMBOLS

Symbol	Explanation	Symbol	Explanation
RUO	For research use only	REF	Catalog number
LOT	Batch number		Consult instructions for use
	Manufacturer		Use by Date
	Temperature limitation		Date of Manufacture
	Contains sufficient for <n> tests		

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