IntelliPlex™ EGFR Mutation Kit User Manual

REF 82006 24 Reactions

RUO For Research Use Only

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IMPORTANT:
Read the instructions carefully prior to use

1. INTENDED USE

The IntelliPlex EGFR Mutation Kit, based on πCode™ technology and PlexBio's instrument platform, is an in-vitro molecular assay intended for qualitative identification of 40 nucleotide changes on exons 12, 18, 19, 20 and 21 of the EGFR gene using DNA samples derived from formalin-fixed paraffin-embedded (FFPE) of non-small cell lung cancer (NSCLC) tissue. Results are intended to assist clinician in identifying NSCLC patients who may benefit from treatment with receptor tyrosine kinase inhibitors like Erlotinib.

2. INTRODUCTION

Epidermal growth factor receptor (EGFR) is a cell surface receptor-tyrosine kinase involved in the intracellular signaling cascades including the PI3K-AKT-mTOR and RAS-RAF-MEK-ERK Pathways. Many types of cancers are associated with mutations in the EGFR gene. Mutations are often found in exons 18, 19, 20 and 21, which encode several regions of the kinase domain. Some mutations cause gene amplification, while others cause increased kinase activity. In any case, these mutations upregulate EGFR activity, leading to enhanced cell survival and proliferation.

Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) such as Erlotinib (orally active) has commonly been used as a therapeutic option in non-small-cell lung cancer (NSCLC) patients. It has been demonstrated efficacy in patients particularly harboring activating mutations of the EGFR gene.

It is thus critical to assess the mutation status of the EGFR gene. Detection of 40 mutations of the EGFR gene per reaction in the background of wild-type genomic DNA in specimens is feasible based on the SelectAmp and πCode technology. The IntelliPlex EGFR Mutation Kit will identify 40 nucleotide changes on exons 12, 18, 19, 20 and 21 of the EGFR gene, as listed in Table 1.

Table 1: Mutations of the EGFR gene

Exon	Amino Acid Change	Nucleotide Change	Cosmic ID
12	S492R	c.1476C>A	236670
18	G719S	c.2155G>A	6252
	G719C	c.2155G>T	6253
	G719A	c.2156G>C	6239
19	K745_E749del	c.2233_2247del15	26038
	E746_S752>D	c.2238_2255del18	6220
	E746_S752>A	c.2237_2254del18	12367
	E746_S752>V	c.2237_2255>T	12384
	E746_T751del	c.2236_2253del18	12728
	E746_T751>A	c.2237_2251del15	12678
	E746_T751>V	c.2237_2252>T	12386
	E746_T751>I	c.2235_2252>AAT	13551
	E746_A750del	c.2235_2249del15	6223
	E746_A750del	c.2236_2250del15	6225
	E746_A750>IP	c.2235_2248>AATTC	13550
	E746_T751>IP	c.2235_2251>AATTC	13552
	E746_S752>I	c.2235_2255>AAT	12385
	E746_T751>VA	c.2237_2253>TTGCT	12416
	E746_P753>VS	c.2237_2257>TCT	18427
	L747_S752del	c.2239_2256del18	6255
	L747_P753>S	c.2240_2257del18	12370
	L747_P753>Q	c.2239_2258>CA	12387
	L747_T751del	c.2239_2253del15	6254
	L747_T751del	c.2240_2254del15	12369
	L747_T751del	c.2238_2252del15	23571
	L747_T751>P	c.2239_2251>C	12383
	L747_T751>S	c.2240_2251del12	6210
	L747_T751>Q	c.2238_2252>GCA	12419
	L747_A750>P	c.2238_2248>GC	12422

	L747_A750>P	c.2239_2248TTAAGAGAAG>C	12382
	L747_E749del	c.2239_2247del9	6218
	L747_S752>Q	c.2239_2256>CAA	12403
20	T790M	c.2369C>T	6240
	S768I	c.2303G>T	6241
	V769_D770insASV	c.2307_2308ins9	12376
	H773_V774insH	c.2319_2320insCAC	12377
	D770_N771insG	c.2310_2311insGGT	12378
21	L858R	c.2573T>G	6224
	L858R	c.2573_2574TG>GT	12429
	L861Q	c.2582T>A	6213

3. TECHNOLOGICAL PRINCIPLES

The IntelliPlex EGFR Mutation Kit utilizes two technologies- SelectAmp and πCode - for detection of 40 EGFR gene mutations in one well 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 well 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 research use only.
- 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 EGFR Mutation Kit contains sufficient reagents for up to 24 tests. The kit components supplied are listed as follows.

1. EGFR KIT Reaction Mix
Ref. No.: 20080
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. **EGFR KIT Primer Mix**
   Ref. No.: 20081
   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. **EGFR KIT πCode MicroDisc**
   Ref. No.: 20084
   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. **EGFR KIT POS Control**
   Ref. No.: 20082
   Quantity & Volume: 1 vial, 20 µL/vial
   Description: Assay positive control
   Contents: EGFR L858R plasmid DNA (Microbial)
   Tris-EDTA Buffer

5. **EGFR KIT NEG Control**
   Ref. No.: 20083
   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. **EGFR KIT Hy Buffer**
   Ref. No.: 20086
   Quantity & Volume: 1 vial, 2.4 mL/vial
   Description: For hybridization
   Contents: Saline-Sodium Phosphate-EDTA

8. **EGFR KIT 10X Wash Buffer**
   Ref. No.: 20088
   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™ RU (PlexBio; Cat. No. 80050)

7. STORAGE, STABILITY AND TRANSPORTATION

Storage

All kit components of the IntelliPlex EGFR Mutation Kit should be stored at 2°C to 8°C.

Stability

Do not use the IntelliPlex EGFR 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 EGFR 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:

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

KIT APP Installation

1. Open the EGFR 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. Using the barcode scanner to read the barcoded ENC file that printed on the inside top lid of the kit.
2. The ENC file will be automatically imported into DeXipher.
3. Make sure the information matches the lot no. of the assay kit then the assay is ready to operate.

9. SPECIMENS

Specimen Collection

The non-small-cell lung cancer (NSCLC) formalin-fixed paraffin embedded (FFPE) tissue have been validated to use with the IntelliPlex EGFR 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 at 15-30°C for over 12 months.

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 EGFR 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
EGFR Reaction Mix	11
EGFR 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

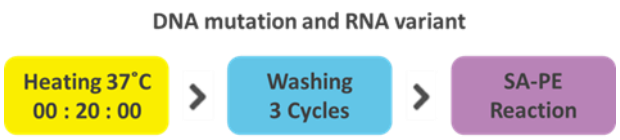
1. Transfer the 10X Wash Buffer to 1L Wash Buffer bottle of the IntelliPlex 1000 πCode Processor supplied and add 450 ml ddH2O to prepare 1X Wash Buffer for use.
2. Pipet the desired volume of SA-PE solution into SA-PE solution tank (V-tray). Please note that the dead volume of V-tray is **500 µL** and 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. Vortex mix the EGFR πCode MicroDisc for 10 seconds, and add 20 µL of EGFR πCode MicroDisc to each well directly without further pipetting. Vortex the EGFR πCode MicroDisc every 4 wells to ensure homogeneous suspension of the EGFR πCode MicroDisc.
4. Add 100 µL of EGFR 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.

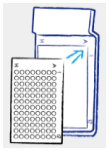


***Note:**

- ***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 that the nucleotide changes in exons 12, 18, 19, 20, and 21 of the EGFR gene is not detected by IntelliPlex EGFR Mutation Kit. It does not preclude the nucleotide changes in exons 12, 18, 19, 20, and 21 of the EGFR 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 nucleotide changes in exons 12, 18, 19, 20, and 21 of the EGFR gene are detected by IntelliPlex EGFR Mutation Kit. It does not preclude the possibility that the specimen did not have mutation in exons 12, 18, 19, 20, and 21 of the EGFR 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	Refer to Table 1.	Mutation detected on the targeted EGFR regions
Mutation Not Detected	None	Mutation not detected on the targeted EGFR 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 EGFR cell line (K562) across 3 days and duplicates of 9 wild-type EGFR 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 9 FFPE specimens were obtained from IRB approval of MacKay Memorial Hospital. Each FFPE specimens were confirmed as EGFR wild-type specimens by Sanger sequencing or Pyrosequencing before LoB determination tests.

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

Limit of Detection (LoD)

The limit of detection (LoD) of IntelliPlex EGFR Mutation Kit was determined for 40 nucleotide changes on exons 12, 18, 19, 20 and 21 of the EGFR gene. Each mutant DNA was tested from 5 different mutation levels of plasmid blended with wild-type EGFR cell line DNA (K562). The mutation level range are serial diluted and DNA from EGFR wild-type FFPE as 0%. The LoD was performed by using 2 lots of IntelliPlex EGFR 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 EGFR Mutation

Mutation (Cosmic ID)	Final LoD (%)	Mutation (Cosmic ID)	Final LoD (%)
236670	0.5	12387	0.5
6252	0.57	12367	0.5
6253	0.58	12384	0.5
6239	2.3	12728	0.5
6240	0.8	12678	0.5
6241	2.9	6254	0.5
12376	1.4	12386	0.5
12377	2	13551	1.5
12378	2.4	26038	0.5
6213	0.5	6223	0.5
6224	2.5	6225	0.525
12429	1.1	12383	0.5
13552	0.525	6210	0.5
12385	0.5	12419	0.5
12416	0.5	12422	0.5
18427	0.5	12382	0.5
12403	0.5	6218	1.6
6255	0.5	13550	0.5
12370	0.5	6220	0.57

Repeatability and Reproducibility

The repeatability and reproducibility of IntelliPlex EGFR Mutation Kit was evaluated across three reagent lots, 2 operators, 2 sets of instrument and 5 non-consecutive testing days. Four replicate runs were performed per reagent lot per day for a total of 40 valid runs at one site. Repeatability of IntelliPlex EGFR Mutation Kit was demonstrated with low level mutant (2x LoD) and high level mutant (6x LoD). The accuracy of the all testing level was at least 92.5% (37/40) across all variance combined (i.e., site/instrument, operator, and day).

Table 6. Repeatability and Reproducibility Accuracy of Each EGFR Mutation

Mutation (Cosmic ID)	Mutation Level (%)	Mutation Detected	Mutation Not Detected	Accuracy (%)
236670	6X	40	0	100%
	2X	40	0	100%
6252	6X	40	0	100%
	2X	39	1	97.5%
6253	6X	40	0	100%
	2X	39	1	97.5%
6239	6X	40	0	100%
	2X	40	0	100%
26038	6X	40	0	100%
	2X	40	0	100%
6220	6X	40	0	100%
	2X	39	1	97.5%
12367	6X	40	0	100%
	2X	40	0	100%
12384	6X	40	0	100%
	2X	40	0	100%
12728	6X	40	0	100%
	2X	39	1	97.5%

12678	6X	40	0	100%
	2X	39	1	97.5%
12386	6X	40	0	100%
	2X	40	0	100%
13551	6X	40	0	100%
	2X	37	3	92.5%
6223	6X	40	0	100%
	2X	40	0	100%
6225	6X	39	1	97.5%
	2X	39	1	97.5%
13550	6X	40	0	100%
	2X	40	0	100%
13552	6X	40	0	100%
	2X	40	0	100%
12385	6X	40	0	100%
	2X	40	0	100%
12416	6X	40	0	100%
	2X	40	0	100%
18427	6X	40	0	100%
	2X	39	1	97.5%
6255	6X	40	0	100%
	2X	40	0	100%
12370	6X	40	0	100%
	2X	39	1	97.5%
12387	6X	40	0	100%
	2X	39	1	97.5%
6254/ 12369/ 23571	6X	40	0	100%
	2X	39	1	97.5%
12383	6X	40	0	100%
	2X	40	0	100%
6210	6X	40	0	100%
	2X	40	0	100%
12419	6X	40	0	100%
	2X	40	0	100%
12422	6X	40	0	100%
	2X	39	1	97.5%
12382	6X	40	0	100%
	2X	40	0	100%
6218	6X	40	0	100%
	2X	40	0	100%
12403	6X	40	0	100%
	2X	40	0	100%
6240	6X	40	0	100%
	2X	40	0	100%
6241	6X	40	0	100%
	2X	39	1	97.5%
12376	6X	40	0	100%
	2X	39	1	97.5
12377	6X	39	1	97.5%
	2X	38	2	95%
12378	6X	40	0	100%
	2X	37	3	92.5%
6224	6X	40	0	100%
	2X	40	0	100%
12429	6X	40	0	100%
	2X	40	0	100%
6213	6X	40	0	100%
	2X	40	0	100%
Total	**-**	**3017**	**23**	**99.24%**

Cross-Reactivity

The cross-reactivity was evaluated by testing the EGFR homolog plasmids (HER2) to eliminate the false positive results. The tested plasmids were divided into two groups: one was DNA blended with 5% of EGFR mutant (Cosmic ID: 6224) in a background of 95% of HER2 DNA. Another one was tested with 100% of HER2 DNA. Each group was operated with duplicates per day in 2 days testing. The results demonstrated that there was no cross reactivity with any of the tested samples (table 7).

Table 7. Cross Reactivity Tests of EGFR between HER2

HER2 Mutation (Cosmic ID)	Test Group	Total Tests	Test Results	Accuracy %
20959	HER2- 95% EGFR 6224- 5%	4	6224	100%
	HER2- 100%	4	WT	100%
12558	HER2- 95% EGFR 6224- 5%	4	6224	100%
	HER2- 100%	4	WT	100%
12553	HER2- 95% EGFR 6224- 5%	4	6224	100%

	HER2- 100%	4	WT	100%
14062	HER2- 95% EGFR 6224- 5%	4	6224	100%
	HER2- 100%	4	WT	100%
14060	HER2- 95% EGFR 6224- 5%	4	6224	100%
	HER2- 100%	4	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 EGFR L858R 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 EGFR L858R 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 EGFR Mutation Kit.

Table 10. The Tested Interfering Substances

Interfering Substance	Assumed Interference Residue Volume (µL / 20 µL 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
RUO	For research use only	REF	Catalog number
LOT	Batch number		Consult instructions for use
	Manufacturer		Use by Date
	Temperature limitation		Caution
	Contains sufficient for <n> tests		Date of Manufacture

15. REFERENCES

1. *My Cancer Genome.* http://www.mycancergenome.org/content/disease/lung-cancer/egfr/
2. Soh et al. (2009) Oncogene mutations, copy number gains and mutant allele specific imbalance (MASI) frequently occur together in tumor cells. PLoS: 4(10):e7464.
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4. Sgambato et al. (2012) The role of EGFR tyrosine kinase inhibitors in the first-line treatment of advanced non small cell lung cancer patients harboring EGFR mutation. Curr Med Chem. 19(20):3337-52
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