IntelliPlex™ Lung Cancer Panel – cfRNA

REF 82031 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 – cfRNA**, based on πCode™ technology and PlexBio's instrument platform, is an in vitro molecular assay intended for the qualitative identification of 28 gene variants of the ALK, ROS1, RET, NTRK1, and MET genes using cell-free RNA derived from plasma 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. Furthermore, copy number variations (CNVs) signatures have been developed to differentiate between NSCLC and SCLC, in particular for poorly differentiated NSCLC, to complement current pathology practices using small biopsies or cytology specimens.

Targeted small molecule inhibitors are now available or being developed to benefit specific molecularly defined subsets of NSCLC patients, and assessment of a variety of mutation status of multiple oncogenes has become critical in the evaluation of cancer treatments. One step RT-PCR with πCode technology enables multiplex, single-well detection of gene rearrangements from RNA samples containing large amounts of wild-type RNA with significantly reduced sample requirement compared to conventional methods. The **IntelliPlex Lung Cancer Panel – cfRNA** identifies 28 gene variants from RNA samples (Table 1).

Table 1. Fusion Variants Detected

Gene	Fusion Variant	Inferred Breakpoint
ALK	V1	E13;A20
	V2	E20;A20
	V3a	E6a;A20
	V3b	E6b;A20

Gene	Fusion Variant	Inferred Breakpoint
	V4	E14;A20
	V5a	E2a;A20
	V5b	E2b;A20
	V"5"	E18;A20
ROS1	CD74-ROS1	C6;R32
		C6;R34
	SLC34A2-ROS1	SL4;R32
		SL4;R34
	SDC4-ROS1	SD2;R32
		SD4;R32
		SD4;R34
	EZR-ROS1	E10;R34
	TPM3-ROS1	T8;R35
RET	KIF5B-RET	K15;R11
		K15;R12
		K16;R12
		K22;R12
		K23;R12
	NCOA4-RET	N6;R12
	TRIM33-RET	T14;R12
	CCDC6-RET	C1;R12
NTRK1	CD74-NTRK1	C8;N12
	MPRIP-NTRK1	M21;N14
MET	MET Exon14 skipping	-

3. TECHNOLOGICAL PRINCIPLES

One-step RT-PCR

One-step RT-PCR combines cDNA synthesis and PCR amplification in a single tube, reducing operation time and contamination risk while yielding highly sensitive results.

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. Cell- free RNA extraction from plasma specimens
2. Variants-specific multiplex RT-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.
- **The reconstituted RNA POS Control should be single use. Do not repeatedly freeze-thaw the RNA POS Control.**
- 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.
- **Avoid RNase contamination:**
  - **Prepare an RNase-free working environment.**
  - **Wear gloves during all steps of the procedure.**
  - **Change gloves frequently.**
  - **Use sterile, disposable polypropylene tubes and filter tips.**
  - **Keep tubes closed whenever possible during the preparation.**
  - **Use RNase removing product to clean bench surfaces, pipettes and other components used in the experiment.**
- 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 – cfRNA** 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 – cfRNA** contains sufficient reagents for up to 24 tests. Kit components include:

(1) cLCP-RNA RT-PCR Buffer
Ref. No.: 20493-R
Quantity & Volume: 1 vial, 360 µL/vial
Description: For RT-PCR amplification
Contents: 2X Reaction Mix, $MgSO_4$ and dNTPs

(2) cLCP-RNA RT-PCR Enzyme
Ref. No.: 20492-R
Quantity & Volume: 1 vial, 24 µL/vial
Description: For RT-PCR amplification
Contents: RT/HotStar Taq MIX, Rnase Inhibitor (Ribolock)

(3) cLCP-RNA Primer Mix
Ref. No.: 20491-R
Quantity & Volume: 1 vial, 96 µL/vial
Description: For RT-PCR amplification
Contents: <20 % Forward Primer, <10 % Reverse Primer (biotin labeled)

(4) cLCP-RNA πCode MicroDisc
Ref. No.: 20494-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

(5) cLCP-RNA POS Control
Ref. No.: 20496-R
Quantity & Volume: 3 vials, lyophilized
Description: Assay positive control; reconstituted with 50 µL nuclease-free water per vial prior to use.
Contents: Cell line RNA, 80 % RNAstable®

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

(7) cLCP-RNA Hy Buffer
Ref. No.: 20495-R
Quantity & Volume: 1 bottle, 2.4 mL/bottle
Description: For hybridization
Contents: Saline-Sodium Phosphate-EDTA

(8) ddH_2O
Ref. No.: 20548-R
Quantity & Volume: 1 vial, 1.5 mL/vial
Description: for reconstitution of cLCP-RNA POS Control
Contents: Nuclease-free water

(9) 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) Blood Collection Tube: RNA Complete BCT®(Streck, Cat. No. 230460, 230461, 230462) or Vactainer® Venous Blood Collection Tube (BD, Cat. No. 367525)
- (Recommended) RNA extraction kit: (QIAamp exoRNeasy Maxi Kit (50), Qiagen Cat. No. 77164 or equivalent)
- Qubit™ Fluorometer with dedicated quantitative reagents (Qubit™ microRNA Assay Kit, Invitrogen; Cat. No. Q32880 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 **cLCP-RNA RT-PCR Buffer** and **cLCP-RNA RT-PCR Enzyme** of the IntelliPlex Lung Cancer Panel – cfRNA should be stored at -15°C to -25°C separately upon arrival.

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

Reconstituted cLCP-RNA POS Control should be single use. Do not repeatedly freeze-thaw the RNA POS Control.

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 – cfRNA** 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-cfDNA-cfRNA 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-cfDNA-cfRNA** 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 file.

10. SPECIMENS

Specimen Collection

The **IntelliPlex Lung Cancer Panel – cfRNA** has been validated to be used for plasma. It is recommended to collect whole blood sample with Streck RNA Complete BCT®(Cat. No. 230460, 230461, 230462) or BD Vactainer® Venous Blood Collection Tube (Cat. No. 367525). Heparin is not recommended as it may interfere with RT-PCR.

Whole Blood Transportation and Storage

Blood samples in Streck RNA Complete BCT® can be transported at room temperature and stored within 2 days at room temperature according to the product specification.

Blood samples in BD Vacutainer® Venous Blood Collection Tubes can be transported at room temperature or 4°C and process within 1 hour.

Plasma Isolation for cfRNA extraction

To separate plasma from whole blood (8~9 mL), centrifuge the Streck RNA Complete BCT® or BD Vacutainer® Venous Blood Collection Tubes at 1,600 x g for 10 minutes at room temperature.

Transfer upper plasma layer (~4 mL) to a conical tube (not provided). Filtrate the plasma with a 0.8 µm filter (not provided) to a new conical tube, then store in -80 °C for up to 6 months. Plasma should be transported with dry ice that keep plasma frozen during the transportation. Store plasma at -80°C immediately upon arrival.

Storage of Extracted RNA

Extracted RNA can be stored at -20°C for immediate use (≤ 24 hours), or at -80°C for long-term (1 – 14 days) storage. Do not subject the extracted RNA 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 exoRNeasy Maxi Kit to extract cell-free RNA, please follow the extraction kit instructions and ensure that you have at least 10 µL of extracted RNA (≥ 1 ng/µL) ready for analysis.

12. ASSAY PROCEDURE

12.1 RNA Quantification

1. Follow the QIAamp exoRNeasy Maxi Kit instructions to purify cell-free RNA and store the extracted samples properly.
2. Quantify the extracted RNA using a Qubit Fluorometer with dedicated quantitative reagents (or equivalent) according to the manufacturer's protocol.
3. The RNA Stock concentration should be ≥ 1 ng/µL to ensure optimal performance. No need for further dilution of extracted samples, each RT-PCR reaction uses 10 µL of extracted samples, POS control and NEG control to ensure the optimal assay performance.

12.2 Reconstitute cLCP-RNA POS Control:

1. Briefly centrifuge the cLCP-RNA POS Control tube.
2. Add 50 µL of ddH_2O to each required vial of cLCP-RNA POS Control.
3. Make sure the material is fully reconstituted by pipetting up and down several times.

NOTE: Reconstituted POS Control should be single use. Do not repeatedly freeze-thaw the reconstituted POS control.

12.3 Multiplex one-step RT-PCR Amplification

1. Vortex to mix each sample before use.
2. Spin down and keep samples on ice.
3. Prepare the one step RT-PCR Reaction:

For each RT-PCR reaction:

cLCP-RNA RT-PCR buffer	15 µL
cLCP-RNA RT-PCR Enzyme	1 µL
cLCP-RNA Primer Mix	4 µL
Sample/ POS Control/ NEG Control	10 µL
Total	30 µL

NOTE:

- The amount of one-step RT-PCR reagent 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.

4. Mix by tapping the tubes and spin down before placing the tubes on the thermocycler. Set up the one-step RT-PCR program conditions as below:

RT-PCR Program Conditions*

Temp. (˚C)	Duration	Cycles
55	15 min	1
95	2 min	1
95	15 sec	45
60	30 sec	
72	30 sec	
4	Hold	1

NOTE: *Ramp rate: 3.0°C/sec (ABI MiniAmp™; Cat. No. A37834).*

12.4 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 20uL cLCP – RNA πCode MicroDisc to 96 well plate:** Mix the tube of πCode by vortexing for 10 seconds, then, by pipetting, add 20 µL 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 – RNA 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 10 µL of the denatured PCR products to each well and place the 96-well plate into the IntelliPlex 1000 πCode Processor.

7. **Pipet the desired volume of SA-PE solution** into the V Tray in SA-PE tank. Please note that the dead volume of the V Tray is 500 µL for up to 6 selected lanes or 800 µL if more than 6 lanes are selected. The minimum usage unit 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 as described (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 3 runs of 8 samples (including POS and NEG controls) for a maximum of 24 tests.

12.5 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 the **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 variant 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 – cfRNA of the same lot will be required.

13. DISCLAIMERS

Negative Test Result

A negative test result means that the targeted variant was not detected by the kit. It does not preclude a positive result of the targeted variant. 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 variant was detected by the kit. It does not preclude a negative result for the targeted variant. 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
Variant Detected	Refer to Table 1	Targeted variant detected
Variant Not Detected	None	Targeted variant not detected
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.
- "Variant Detected" indicates that the signal for at least one variant 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 performing eight replicates of 47 wild-type plasma specimens on 2 reagent lots and one set of instruments. Origins of the plasma specimens include biobanks from France, US, and Taiwan. The cutoff values of each targeted variants were determined by the measured maximum analytical signal intensity values, respectively.

Only "**Variant 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 5-200 copies) containing different copies of variant RNA. All testing samples were blended in a background of HEK293 cell line RNA. 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 10 to 89 copies.

Table 3. Limit of Detection (LoD)

Gene	Fusion Variant	Inferred Breakpoint	LoD (copies)
ALK	V1	E13;A20	10
	V2	E20;A20	10
	V3a	E6a;A20	10
	V3b	E6b;A20	10
	V4	E14;A20	10
	V5a	E2a;A20	10
	V5b	E2b;A20	10
	V"5"	E18;A20	10
ROS1	CD74-ROS1	C6;R32	89
		C6;R34	14
	SLC34A2-ROS1	SL4;R32	50
		SL4;R34	10
	SDC4-ROS1	SD2;R32	20
		SD4;R32	54
		SD4;R34	10
	EZR-ROS1	E10;R34	10
	TPM3-ROS1	T8;R35	10
RET	KIF5B-RET	K15;R11	10
RET	KIF5B-RET	K15;R12	10
		K16;R12	10
		K22;R12	10
		K23;R12	10
	NCOA4-RET	N6;R12	10
	TRIM33-RET	T14;R12	10
	CCDC6-RET	C1;R12	10
NTRK1	CD74-NTRK1	C8;N12	46
	MPRIP-NTRK1	M21;N14	10
MET	Exon14 skipping	-	40

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/ NEG Control added. Or POS Control not reconstituted properly. 2. RNase contamination. 3. Assay did not work. 4. Cross contamination between samples. 5. Wrong PC/NC wells chose.	1. Ensure POS Control reconstituted properly and do not repeatedly freeze-thaw POS control. Ensure POS and NEG Control are added. 2. Ensure all operating procedures are followed correctly. Ensure work environment is free of RNase. 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.
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. 2. Not enough πCode MicroDiscs added to well. 3. Microbes exist in buffers. 4. Instruments error or malfunction.	1. Re-disperse the microplate using IntelliPlex 1000 Processor, and repeat reading. 2. Ensure πCode MicroDiscs are well-mixed with proper amount added. 3. Use freshly prepared assay wash buffer and deionized water for hybridization to reduce πCode MicroDiscs loss rate. 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. 2. SA-PE solution inactivation. 3. Incorrect tested lanes of microplate selected for SA-PE solution dispensing.	1. Make sure all the assay procedures are followed correctly. Calculate sufficient SA-PE solution volume for dispensing. Repeat test. 2. Ensure correct storage condition and minimize the light exposure. Do not use SA-PE past its expiration date. 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. 2. Residues of SA-PE solution in wells after hybridization. 3. PlexBio 100 Fluorescent Analyzer is not calibrated. 4. Markings on plates.	1. Check correct hybridization program is selected. 2. Ensure all buffers (Assay Wash Buffer and deionized water) on IntelliPlex 1000 Processor are fresh-made and sufficient for washing procedures. 3. Perform calibration on PlexBio 100 Fluorescent Analyzer. 4. Do not make any marking on plate.

Problem	Possible Cause	Recommendations
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. RNase contamination.	3. Ensure all the operating procedures are followed correctly. Ensure work environment is free of RNase.
	4. Hybridization did not work.	4. Make sure all the assay procedures are followed correctly. Ensure samples are freshly heat-denatured.
Reference Gene Fail	Reference Gene monitors quality of tested sample and must be positive.	
	1. No Sample added or absence of human-derived RNA.	1. Ensure human-derived RNA samples are added. Do not use artificial RNA 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 and RIN (RNA integrity number) value. 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		Caution

18. REFERENCES

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