



IntelliPlex™ BRAF V600 Mutation Kit

REF 82004 24 Reactions

RUO For Research Use Only



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IMPORTANT:
Read the instructions carefully prior to use

1. INTENDED USE

The IntelliPlex BRAF V600 Mutation Kit, based on π Code™ technology and PlexBio's instrument platform, is an in-vitro molecular assay intended for qualitative identification of 7 nucleotide changes in exon 15 of the *BRAF* gene from a variety of suitable samples, including DNA samples derived from formalin-fixed paraffin-embedded (FFPE) tissue of melanoma, non-small cell lung cancer (NSCLC) or metastatic colorectal cancer (mCRC). This product is for research use only, and not for use in diagnostic procedures.

2. INTRODUCTION

The mitogen-activated protein kinase (MAPK) pathway is an important signaling pathway in a number of cancers. Activating mutations in *BRAF* result in constitutive activation of the RAF-MEK-ERK signaling cascade, leading to unchecked cell proliferation and growth. Many types of cancers, including melanoma, NSCLC, and mCRC, are associated with activating mutations in the *BRAF* gene, often found in exon 15 of codon 600. SelectAmp and π Code technology enables the multiplex, single-well detection of single nucleotide mutations of the *BRAF* gene from specimens containing large amounts of wild-type genomic DNA with significantly reduced sample requirement compared to conventional methods. The IntelliPlex BRAF V600 Mutation Kit identifies 7

nucleotide changes in exon 15 of the *BRAF* gene (Table 1).

Table 1. Mutations Detected

Gene	Exon Codon	Amino Acid Change	Nucleotide Change	Cosmic ID
BRAF	Exon 15 Codon 600	p.V600E1	c.1799T>A	476
		p.V600E2	c.1799_1800TG>AA	475
		p.V600D	c.1799_1800TG>AT	477
		p.V600G	c.1799T>G	6137
		p.V600K	c.1798_1799GT>AA	473
		p.V600R	c.1798_1799GT>AG	474
		p.V600M	c.1798G>A	1130

3. TECHNOLOGICAL PRINCIPLES

The IntelliPlex BRAF V600 Mutation Kit utilizes two technologies, SelectAmp and π Code, to achieve high sensitivity multiplex mutation detection.

SelectAmp Technology

SelectAmp technology enables mutation-specific multiplex PCR amplification by blocking amplification of wild-type sequences with Locked Nucleic Acid (LNA). The subsequent selective PCR amplification of mutated sequences increases assay sensitivity and specificity.

π Code MicroDisc

π Code MicroDisc are manufactured to generate up to 16,000 distinct circular image patterns for multiplexing applications. Each π Code MicroDisc has a distinct circular image pattern, which corresponds to a specific capture agent conjugated to the surface of the disc. π Code 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 specimens
2. Mutation-specific multiplex PCR amplification
3. Hybridization of PCR amplicons with mutation-specific probe tagged π Code 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.
- Note that tumor samples are non-homogeneous in terms of genotype, and may contain non-tumor sections, which can cause false negative results.
- 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 (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. Kit components include:

- 1. BRAF KIT Reaction Mix**
Ref. No.: 20097-R
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 KIT Primer Mix**
Ref. No.: 20098-R
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 KIT πCode MicroDisc**
Ref. No.: 20099-R
Quantity & Volume: 1 vial, 480 µL/vial
Description: For PCR amplicon capture
Contents: πCode MicroDisc, Glycerol Phosphate buffered saline, 0.1% Albumin- from bovine (Biological), <0.1% EDTA and <0.1% Sodium azide
- 4. BRAF KIT POS Control**
Ref. No.: 20101-R
Quantity & Volume: 1 vial, 20 µL/vial
Description: Assay positive control
Contents: <0.001% BRAF plasmid DNA (Microbial), Tris-EDTA Buffer
- 5. NEG Control**
Ref. No.: 20549-R
Quantity & Volume: 1 vial, 120 µL/vial
Description: Assay negative control
Contents: ddH₂O
- 6. 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. Hy Buffer**Ref. No.:** 20547-R**Quantity & Volume:** 1 bottle, 2.4 mL/bottle**Description:** For hybridization**Contents:** Saline-Sodium Phosphate-EDTA**8. 10X Wash Buffer****Ref. No.:** 20546-R**Quantity & Volume:** 1 bottle, 50 mL/bottle**Description:** For π Code washing**Contents:** Phosphate buffered saline,
1% Tween-20 and <0.1% Sodium azide

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

6. 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 π Code 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)

Required components:

- 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
- ddH₂O for dilution of 10X Wash Buffer
- Nucleic acid sample purification kit compatible with downstream PCR amplification
- For FFPE samples: FFPE DNA extraction kit (Recommended: QIAamp DNA FFPE Tissue Kit, Qiagen; Cat. No. 56404 or equivalent)
- Vortex mixer
- Micro-centrifuge
- Thermocycler (Recommended: DigiPlex™ Thermocycler, PlexBio; Cat. No. 80018/ MiniAmp™ Thermal Cycler, Applied Biosystems™; Cat. No. A37834 or equivalent)
- Industrial Computer (Recommended: PlexBio; Cat. No. 80002)

7. STORAGE, STABILITY AND TRANSPORTATION**Storage**

All kit components should be stored at 2-8°C.

Stability

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

8. INSTRUMENT AND SOFTWARE**Instrument**Please refer to the instrument user manual for complete operation instructions (Thermocycler, IntelliPlex 1000 π Code Processor and PlexBio 100 Fluorescent Analyzer).**Software Installation**The BRAF V600 Mutation Kit has a designated Kit App and ENC file. The Kit App contains the π Code 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. Log into www.plexbio.com and download the **BRAF V600 Mutation Kit 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. Log into www.plexbio.com and download the **BRAF V600 Mutation Kit 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 RUO kit.
2. Save the ENC file to your computer.
3. Follow the PlexBio 100 Fluorescent Analyzer User Manual to import the ENC file.

9. SAMPLES

The **IntelliPlex BRAF V600 Mutation Kit** supports a variety of samples, including the use of formalin-fixed paraffin embedded tissue (FFPET). The nucleic acid of all samples must be extracted with a purification procedure supporting downstream PCR amplification.

NOTE FOR USE OF FFPET:

- FFPET specimens may be stored $\leq 30^{\circ}\text{C}$ 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 BRAF 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.
- The presence of melanin (PCR inhibitor) may cause a false negative result or an invalid result. When melanin inhibition is suspected, repeat testing on an unpigmented FFPE tissue section.

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.

10. BEFORE YOU START

- Check that the Kit App has been installed and the lot specific ENC file has been imported to DeXipher.
- Check that you have 4 μL of extracted DNA (≥ 2.5 ng/ μL) ready for analysis.

11. ASSAY PROCEDURE

Warning:

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

11.1 DNA Quantification

- Quantify the extracted DNA using a Qubit Fluorometer with dedicated quantitative reagents (or equivalent) according to the manufacturer's protocol.
- The DNA stock concentration should be ≥ 2.5 ng/ μL to ensure optimal assay performance. Each PCR reaction uses 4 μL of a 2.5 ng/ μL DNA working stock (10 ng DNA input). Please prepare working stock for

all samples before preparing PCR. DNA input amounts lower or higher than 10 ng per reaction are not recommended.

11.2 Multiplex PCR Amplification

- Vortex to mix each sample before use.
- Spin down and keep samples on ice.
- Prepare the PCR Reaction:

For each PCR reaction:

BRAF Reaction Mix	11 μL
BRAF Primer Mix	5 μL
Sample/POS Control/NEG Control	4 μL
Total volume	20 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.
- Mix by tapping the tubes and spin down before placing the tubes on the thermocycler. Set up the PCR program conditions as below:

PCR Program Conditions*

Temp. ($^{\circ}\text{C}$)	Time	Cycles
95	5 min	-
95	20 sec	36
70	20 sec	
60	60 sec	
4	Hold	-

NOTE: Ramp rate: 20% (PlexBio; Cat. No. 80018).

3 $^{\circ}\text{C}/\text{sec}$ (ABI MiniAmpTM; Cat. No. A37834).

11.3 DNA Hybridization and SA-PE Reaction

- Prepare 1X Wash Buffer:** Transfer 50mL of the 10X Wash Buffer to the IntelliPlex 1000 πCode Processor 1L Wash Buffer bottle and add 450 ml ddH₂O. Mix by swirling.

NOTE: The prepared 1X Wash Buffer can be used for up to one week.

IntelliPlex 1000 π Code Processor Wash Buffer consumption:

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

2. **Add 20 μ L π Code MicroDisc to 96 well plate:** Mix by vortexing the **BRAF π Code** for 10 seconds, then, by pipetting, add 20 μ L of the π Code to each well directly. Vortex the tube of π Code 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 μ L of 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 5 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 μ L of the denatured PCR products** to each well.
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 μ L** for up to 6 selected lanes or **800 μ L** if more than 6 lanes are selected. The minimum usage of SA-PE is **one lane (900 μ L)**.

Calculation Example:

For a 3-lane reaction, the required SA-PE solution volume is at least:

$$400 \mu\text{L} \times 3 \text{ lanes} + 500 \mu\text{L}(\text{dead volume}) = 1.7 \text{ mL}$$

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 (μ L)
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 π Code Processor. Refer to the IntelliPlex 1000 π Code Processor user 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 π Code Processor must be maintained properly and regularly.
- **Do not** open the door when the instrument is in operation.
- The kit contains sufficient reagents for 5 runs of tests (including POS and NEG controls) for a maximum of 24 tests. Please note that the included Wash Buffer is only sufficient for up to two independent runs. Additional Wash buffer can be ordered from PlexBio (Ref. No: 80210).

11.4 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 the **Qualitative Assay**.
3. Mark the wells for sample, positive and negative controls.
4. Enter sample information and assay name. 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 BRAF V600 Mutation Kits of the same lot will be required.
- The procedure described above must be followed to detect $\geq 0.29\sim 1.57\%$ mutant sequences in a background of wild-type DNA for the BRAF mutations in Table 1.

12. DISCLAIMERS

Negative Test Result

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

13. INTERPRETATION OF RESULTS

Table 2. Interpretation of Result

Test Result	Reported Result	Interpretation
Mutation Detected	Ex. V600E1 (Refer to Table 1 for details)	Targeted mutation detected
Mutation Not Detected	None	Targeted mutation not detected

Test Result	Reported Result	Interpretation
Invalid Assay	Invalid	<p>Possible Causes:</p> <ol style="list-style-type: none"> 1. PCR Inhibition (presence of inhibitor in the sample) 2. Improper stored reagents 3. Low sample DNA input or quality 4. Low πCode Disc Count (the πCode tube was not vortexed before pipetting) 5. Reagent not added 6. Failed Blank πCode Control 7. Sample quality due to improper fixation process or storage condition

NOTE:

In case of heterogeneity of samples or multiple mutations, only the dominantly detected mutation is reported. "Mutation Detected" indicates that the signal for at least one mutation site is greater than the cutoff value of the corresponding target. When multiple mutations are detected in a sample, only the one that exhibits the highest signal is reported.

14. TROUBLESHOOTING

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

Problem	Possible Cause	Recommendations
No Valid Assay Assigned	<ol style="list-style-type: none"> 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. 	<ol style="list-style-type: none"> 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.











Problem	Possible Cause	Recommendations
Positive Control Fail / Negative Control Fail	1. No POS Control or NEG Control added.	1. Ensure POS Control and NEG Control are added.
	2. DNase contamination.	2. Ensure all operating procedures are followed correctly. Ensure work environment is free of DNase.
	3. Assay did not work.	3. Make sure all the assay procedures are followed correctly.
	4. Cross contamination between samples.	4. Clean all surfaces and equipment. Operate pre-PCR and post-PCR in the dedicated area and separate the equipment for use.
	5. Wrong PC/NC wells chose.	5. Choose the correct PC/NC wells and repeat reading.
πCode MicroDiscs Count Fail	DeXipher is unable to detect sufficient πCode MicroDiscs numbers for mutation determination.	
	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 wash buffer and ddH ₂ O 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.

Problem	Possible Cause	Recommendations
	2. No SA-PE was added or insufficient SA-PE solution for dispensing.	2. Make sure all the assay procedures are followed correctly. Calculate sufficient SA-PE solution volume for dispensing. Repeat test.
	3. SA-PE solution inactivation.	3. Ensure correct storage condition and minimize the light exposure. Do not use SA-PE past its expiration date.
	4. Incorrect tested lanes of microplate selected for SA-PE solution dispensing.	4. Repeat assay and make sure lanes selected correctly.
	“Background” is determined by measuring MFI of an internal control that should not give a signal.	
Blank Control Fail	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 (Wash buffer and ddH ₂ O) 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.

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. 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 and quality. 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.


15. SYMBOLS

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

Notice to User

The use of this product and the associated PlexBio instrumentation is covered by one or more issued (US10302640B2, US10436778B2, US10436776B2, US9063044B2, US10019815B2) 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. 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.

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