AmoyDx® IDH1/2 PCR Panel

Instructions for Use

For Research Use Only. Not for use in diagnostic procedures.

REF	8.01.0320	10 tests/kit	For SLAN-96S
	8.01.0321	10 tests/kit	For QuantStudio 5
	8.01.0322	10 tests/kit	For LightCycler480 II, cobas z 480

Amoy Diagnostics Co., Ltd.

No. 39, Dingshan Road, Haicang District,

361027 Xiamen, P. R. China

Tel: +86 592 6806835

Fax: +86 592 6806839

E-mail: sales@amoydx.com

Website: www.amoydx.com

Version: P1.0

Mar 2025

Background

IDH1 and IDH2 are two isoforms of isocitrate dehydrogenase that perform crucial roles in cellular metabolism. Somatic mutations in either of these two genes impart a neomorphic enzymatic activity upon the encoded enzymes, resulting in the ability to convert α-ketoglutarate (αKG) into the oncometabolite R2-hydroxyglutarate (R2-HG), which can competitively inhibit multiple αKG-dependent dioxygenases. Inhibition of various classes of αKG-dependent dioxygenases results in dramatic epigenetic changes in hematopoietic cells, which has been found to directly impair differentiation [1].

Acute myeloid leukemia (AML) is a genetically heterogeneous hematologic malignancy. It is now recognized that approximately 8% and 12% of AML cases harbor an IDH1 or IDH2 mutation, respectively [2]. Studies have shown that patients with relapsed/refractory or newly diagnosed AML harboring IDH1 mutations can benefit from treatment with IDH1 inhibitors [3,4]. Similarly, patients with relapsed/refractory AML harboring IDH2 mutations can benefit from treatment with IDH2 inhibitors [5].

Gliomas are the most common type of primary brain tumors [6]. IDH mutations are primarily detected in WHO grade II–III tumors and represent a major biomarker with diagnostic, prognostic, and predictive implications [7]. Research has shown that IDH1/2 mutations occur in approximately 70% of low-grade gliomas [8]. In The 2021 WHO Classification of Tumors of the Central Nervous System, a combined molecular and histological approach has been adopted, wherein IDH1 or IDH2 molecular status is directly integrated as a key high-grade classification criterion [9].

On July 20, 2018, the FDA approved TIBSOVO® (ivosidenib), for adult patients with relapsed or refractory acute myeloid leukemia (AML) with a susceptible IDH1 mutation. On May 25, 2022, the FDA approved a supplemental application for TIBSOVO® (ivosidenib) extending the indication in patients with newly diagnosed IDH1-mutated AML in older adults or those with comorbidities to include the combination with azacitidine. On December 1, 2022, the FDA approved REZLIDHIA® (olutasidenib) for adult patients with relapsed or refractory AML with a susceptible IDH1 mutation. On August 01, 2017, the FDA approved IDHIFA® (enasidenib) for adult patients with relapsed or refractory AML with IDH2 mutation. On August 6, 2024, the FDA approved VORANIGO® (vorasidenib) for adult and pediatric patients 12 years and older with Grade 2 astrocytoma or oligodendroglioma with a susceptible IDH1 or IDH2 mutation.

Intended Use

The AmoyDx® IDH1/2 PCR Panel is a qualitative real-time PCR assay developed for the detection of 15 mutations in the IDH1 gene across exon 4, and IDH2 gene across exon 4, using DNA extracted from peripheral blood/bone marrow of acute myeloid leukemia patients or FFPE of glioma patients.

This kit is intended for research use only and must be operated by trained professionals in a controlled laboratory environment.

This kit is compatible with the LightCycler480 II, cobas z 480, QuantStudio 5 and SLAN-96S platforms for testing and data collection. Results could be analyzed manually or using ARAS, a tool to aid in the interpretation of AmoyDx® IDH1/2 PCR Panel.

Principles of the Procedure

The AmoyDx® IDH1/2 PCR Panel integrates ADx-ARMS® and qualitative real-time PCR technology with real-time PCR, utilizing specific primers and multi-fluorescent channel probes (FAM, VIC) to detect *IDH1*/*IDH2* mutations in DNA from bone marrow/ peripheral

blood/FFPE samples. This approach significantly enhances detection coverage and information yield.

The ADx-ARMS® technology refines traditional ARMS methods through optimized primer and probe design. When the 3' end of the primer perfectly matches the mutant template, efficient amplification occurs, generating detectable signals via FAM-labeled fluorescent probes. Conversely, if the 3' end of the primer mismatches the wild-type template, no amplification occurs, effectively eliminating interference from wild-type DNA. This design significantly enhances the specificity and sensitivity of mutation detection.

To minimize false positives caused by PCR product contamination, the PCR amplification system includes UNG (Uracil-N-Glycosylase) enzyme, which selectively cleaves dU-containing PCR fragments, preventing carryover contamination and ensuring reliable results.

Kit Contents

The AmoyDx® IDH1/2 PCR Panel includes IDH Reaction Mix (8-tube strips), IDH Enzyme Mix, IDH Positive Control.

Table 1 Kit Contents

Content	Main Ingredients	Quantity
IDH Reaction Mix	Primers, Probes, Mg^{2+}, dNTPs	12 strips
IDH Enzyme Mix	Taq DNA Polymerase, UNG Enzyme	45μL/tube ×1
IDH Positive Control	Plasmid DNA, Wild-type DNA	250μL/tube ×1

Note:

- *Do not mix reagents from different batches.*

The AmoyDx® IDH1/2 PCR Panel is designed with an 8-tube PCR strip, where each 8-tube strip is used to test a single sample.

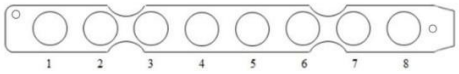
1) Tubes 1-7 contain reagents for detecting 15 mutations in the IDH1/IDH2 genes along with internal control reagents. Mutation detection is indicated by the FAM signal, while the internal control is indicated by the VIC signal.
2) Tube 8 contains reagents for DNA external control, assessing the DNA quality and overall procedure, which is indicated by FAM signal.

Table 2 Composition of the 8-tube Strip

Tube	Target Gene	Fluorescence	Target	Volume
1	IDH1 Exon4	FAM	p.R132S/p.R132H	35 μL
		VIC	Internal Control	
2	IDH1 Exon4	FAM	p.R132C/ p.R132L	35 μL
		VIC	Internal Control	
3	IDH1 Exon4	FAM	p.R132G	35 μL
		VIC	Internal Control	
4	IDH2 Exon4	FAM	p.R140W/ p.R140G/ p.R140Q	35 μL
		VIC	Internal Control	
5	IDH2 Exon4	FAM	p.R140L	35 μL
		VIC	Internal Control	
6	IDH2 Exon4	FAM	p.R172W/ p.R172G/ p.R172M/ p.R172S	35 μL
		VIC	Internal Control	
7	IDH2 Exon4	FAM	p.R172K/ p.R172S	35 μL
		VIC	Internal Control	
8	External Control	FAM	External Control	35 μL

Note:

- *Please distinguish Tube 8 from Tube 1 according to the trapezoid end of the strip edge, as described below.*



Storage and Stability

The kit requires shipment on frozen ice packs below 25℃ for no more than one week. All contents of the kit should be stored immediately upon receipt at -20±5℃ and protected from light.

The shelf-life of the kit is twelve months. Tube opening doesn't affect the expiration of the kit. The recommended maximum freeze-thaw cycle is five cycles.

Additional Reagents and Equipment Required but Not Supplied

1) Compatible PCR instruments: LightCycler480 II, cobas z 480, QuantStudio 5, SLAN-96S
2) Bone marrow/Blood DNA extraction kit: AmoyDx® Blood/Bone Marrow DNA Kit is recommended.
   FFPE extraction kit: AmoyDx® FFPE DNA Kit is recommended.
3) DNA quantification kit: Spectrophotometers, NanoDrop™ 1000/2000 is recommended.
4) Mini centrifuge with rotor for centrifuge tubes.
5) Mini centrifuge with rotor for PCR tubes.
6) Vortexer.
7) Nuclease-free centrifuge tubes.
8) Nuclease-free PCR tubes and caps.
9) Adjustable pipettors and filtered pipette tips for handling DNA.
10) Tube racks.
11) Disposable powder-free gloves.
12) Sterile, nuclease-free water.

Precautions and Handling Requirements

Precautions

- Please read the instruction carefully and become familiar with all components of the kit prior to use, and strictly follow the instruction during operation.
- Please check the compatible real-time PCR instruments prior to use.
- DO NOT use the kit or any kit component after their expiry date.
- DO NOT use any other reagents from different lots in the tests.
- DO NOT use any other reagent in the other test kits.

Safety Information

- Handle all specimens and components of the kit as potentially infectious material using safe laboratory procedures.
- Only trained professionals can use this kit. Please wear suitable lab coat and disposable gloves while handling the reagents.
- Avoid skin, eyes and mucous membranes contact with the chemicals. In case of contact, flush with water immediately.
- DO NOT pipet by mouth.

Decontamination and Disposal

- The kit contains Positive Control; strictly distinguish the Positive Control from other reagents to avoid contamination which may cause false positive.
- PCR amplification is extremely sensitive to cross-contamination. The flow of tubes, racks, pipets and other materials used should be from pre-amplification to post-amplification, and never backwards.
- Gloves should be worn and changed frequently when handling samples and reagents to prevent contamination.
- Using separate, dedicated pipettes and filtered pipette tips when handling samples and reagents to prevent exogenous nucleic acid contamination to the reagents.
- Please pack the post-amplification tubes with two disposable gloves and discard properly. DO NOT open the post-amplification PCR tubes.
- All disposable materials are for one time use. DO NOT reuse.
- The unused reagents, used kit, and waste must be disposed of properly.

Cleaning

- After the experiment, wipe down the work area, spray down the pipettes and equipment with 75% ethanol or 10% hypochlorous acid solution.

Assay Procedure

1. Sample Requirements and DNA Extraction

- The recommended sample types include peripheral blood and bone marrow samples from acute myeloid leukemia (AML) patients or FFPE samples from glioma patients.
- For peripheral blood and bone marrow samples
  1) Peripheral blood and bone marrow samples should be no less than 200 μL, and the use of EDTA anticoagulant is recommended. Please DO NOT use heparin as an anticoagulant.
  2) If DNA extraction is not performed immediately after sample collection, the peripheral blood and bone marrow samples can be stored at 2–8°C or -20±5°C. Storage at 2–8°C should not exceed 7 days, while storage at -20±5°C should not exceed 3 years.
  3) Peripheral blood and bone marrow samples should be transported under constant 2–8°C conditions or with ice-pack for no more than 1 week, and the temperature should not exceed 25°C.
  4) For optimal results, testing should be performed immediately after determining DNA concentrations. It is recommended to

dilute the DNA extracted from peripheral blood or bone marrow samples to 2 ng/μL using 1×TE (pH 8.0).

5) If storage is necessary, keep DNA at -20±5°C for up to 6 months.

- For FFPE samples

1) FFPE samples should contain an adequate proportion of tumor cells (recommended ≥20%; for lower tumor content, enrichment is advised).

2) FFPE samples should be stored for no more than 2 years, with a section thickness of 5–10 μm and at least 5 sections per sample.

3) After DNA extraction, a spectrophotometer (e.g., Nanodrop 1000/2000) is recommended to measure concentration. The ideal concentrations are >2 ng/μL for DNA. If DNA concentration and purity do not meet the requirements, additional sample collection or increasing the sample volume for DNA extraction is recommended.

4) For optimal results, testing should be performed immediately after determining DNA concentrations. The DNA concentration should be adjusted according to the FFPE sample storage time:

FFPE Storage Time	Recommended DNA Concentration
<3 months	1 ng/μL
3-12 months	1.5 ng/μL
1-2 years	1.5–2 ng/μL

- *The DNA loading concentration can be adjusted appropriately based on sample quality, and FFPE samples stored for more than 2 years are not recommended.*

5) If storage is necessary, keep DNA at -20±5°C for up to 6 months.

Note:

- *The No Template Control (NTC, nuclease-free water) should be included in parallel during the nucleic acid extraction process.*

2. DNA Mix Preparation and Plate Setup

In each PCR process, each sample must be tested and analyzed together with a Positive Control (PC) and a No Template Control (NTC, nuclease-free water).

1) Thaw the IDH Reaction Mix (sufficient for samples as well as PC and NTC) and IDH Positive Control.

2) Vortex the above tubes to assure no frozen components exist (10-15 seconds).

3) Spin the above tubes and IDH Enzyme Mix briefly to collect the contents at the bottom of the tubes (10-15 seconds).

4) Place the above tubes into an ice rack.

5) IDH Enzyme Mix should be pre-mixed with samples (as well as control samples of PC and NTC) as Master Mix. The ratio is shown as follows, named S-Mix, P- Mix and N-Mix respectively.

Reagent	Volume per test
Sample (DNA, PC, NTC)	42.3 μL
IDH Enzyme Mix	2.7 μL

6) Mix the Master Mix by vortexing and briefly centrifuge (10-15 seconds).

7) Dispense 5 μL of Master Mix into tubes 1-8.

8) Seal the 8-tube strips with the optical sealing caps.

9) Briefly centrifuge (10-15 seconds) the strips to collect reaction at the bottom of the tubes and to ensure that there are no bubbles at the bottom of the tubes.

10) Place the 8-tube strips into the appropriate positions of the real-time PCR instrument. A recommended plate layout is shown in Figure 1.

Tube 1	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NTC
Tube 2	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NTC
Tube 3	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NTC
Tube 4	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NTC
Tube 5	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NTC
Tube 6	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NTC
Tube 7	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NTC
Tube 8	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NTC

Figure 1 Layout for a Single Test Plate Analyzing 10 Unknown Samples

Note:

- *Each run must contain one PC and one NTC.*
- *The prepared mixtures should be used immediately, avoid prolonged storage.*
- *Due to the viscosity of the enzyme mix, pipet slowly to ensure all mix is completely dispensed from the tip.*
- *Pipet enzyme mix by placing the pipet tip just under the liquid surface to avoid the tip being coated in excess enzyme.*

3. Instrument Setup

Table 4 Real-Time PCR Parameters

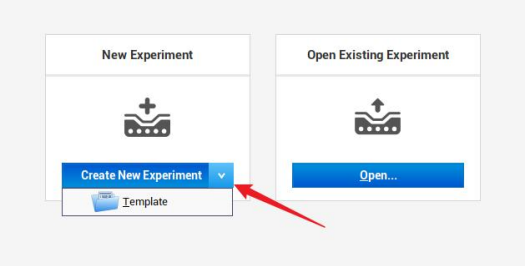
Stage	Cycles	Temperature	Time	Data Collection
1	1	95℃	5 min	/
2	15	95℃	25 s	/
		64℃	20 s	/
		72℃	20 s	/
3	31	93℃	25 s	/
		60℃	35 s	FAM, VIC
		72℃	20 s	/
4	1	40℃	30 s	/

To complete the instrument setup, please follow the provided instructions to import the real-time PCR run configuration file. Ensure all parameters are correctly configured according to the file for optimal performance.

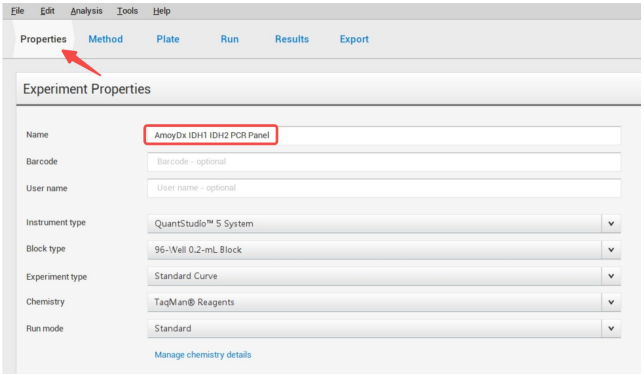
- QuantStudio 5

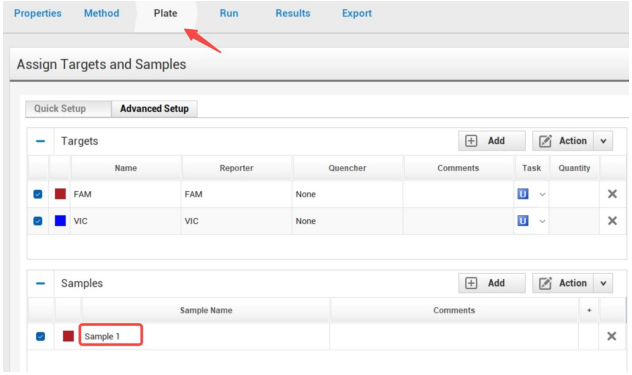
1) Open the QuantStudio 5 software.

2) Click the “arrow” next to the "Create New Experiment" button to upload the template of “AmoyDx IDH1 IDH2 PCR Panel.edt” provided by AmoyDx. If the template is not available, please reach out to your regional Field Application Scientist.



3) Set the experiment name and sample name per your request.





4) Leave other parameters by default.

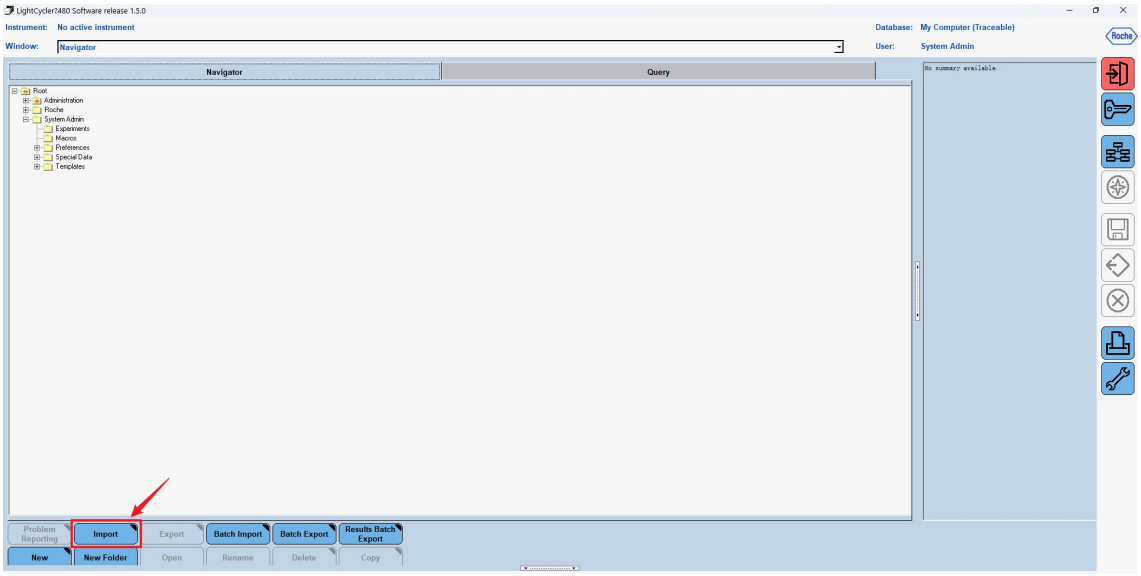
5) Click “START RUN” button to start the PCR process.

- LightCycler480 II or cobas z 480

1) Open the LightCycler480 software.

2) Click “New experiment” in the main page.

3) Click “Import” in the bottom bar.

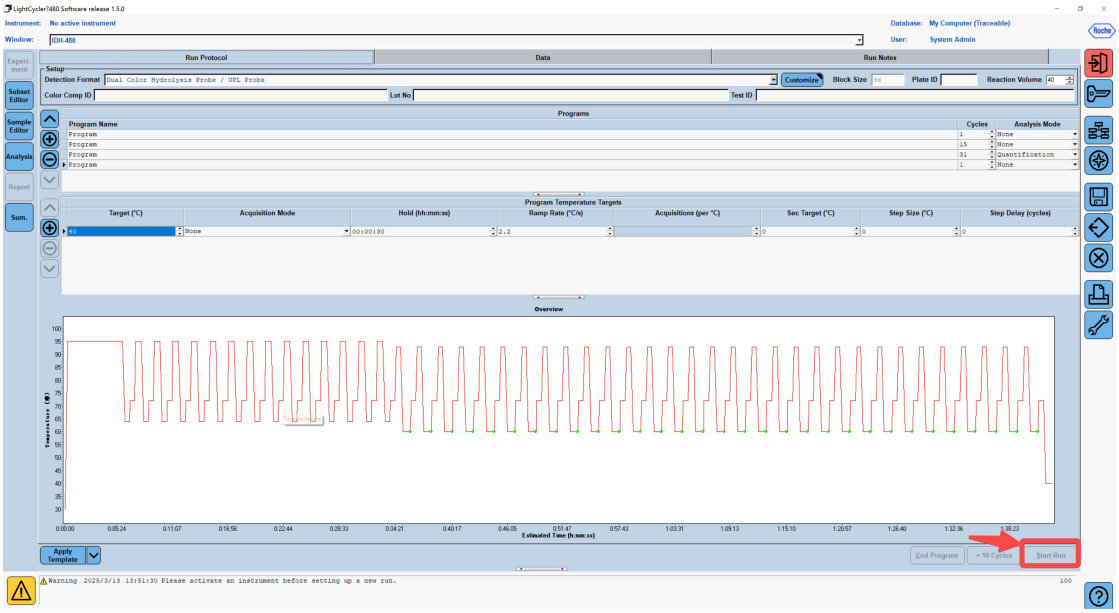


4) Select the appropriate AmoyDx® IDH1/2 PCR Panel run protocol template based on your instrument type:

a) For LightCycler480 II, use “AmoyDx IDH1 IDH2 PCR Panel for LC480.ixo”.

b) For cobas z 480, use “AmoyDx IDH1 IDH2 PCR Panel for cobas Z480.ixo”.

5) Click “Start Run” button to start the PCR process.



6) Click “Sample Editor” button on the left sidebar to enter sample information.

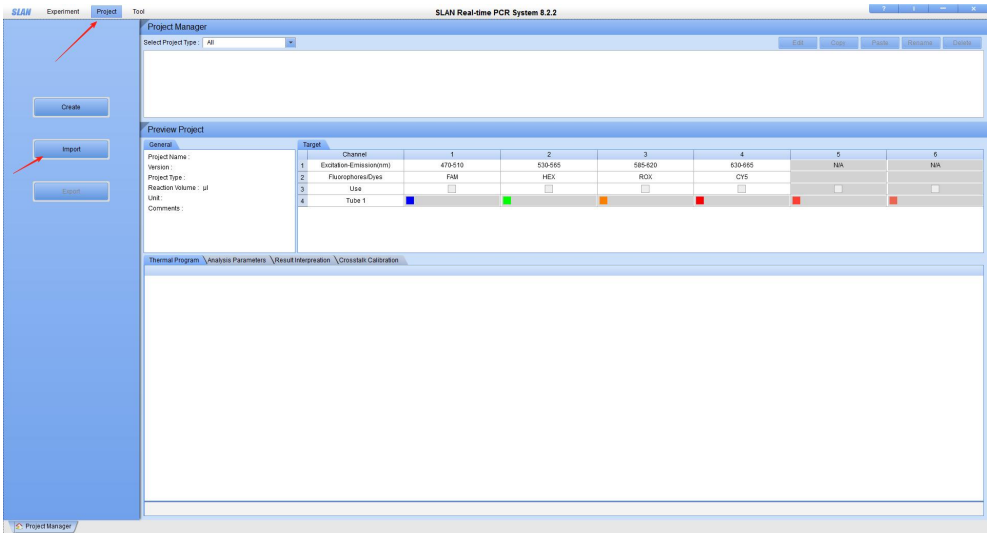
- SLAN-96S

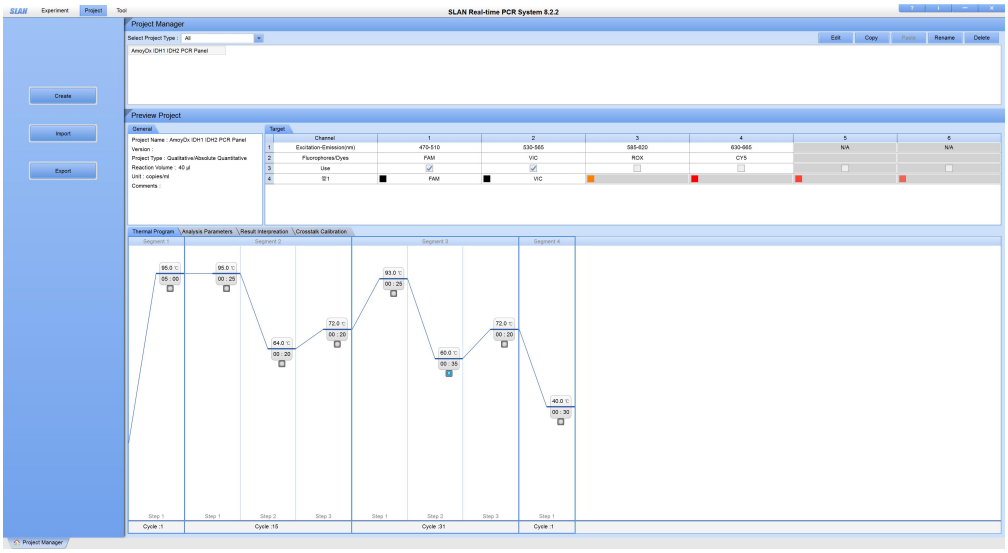
1) Open the SLAN-96S software.

2) Click “Project” in the top bar.

3) Click “Import” in the left sidebar.

4) Select “AmoyDx IDH1 IDH2 PCR Panel.prj” provided by AmoyDx. If the project file is not available, please reach out to your regional Field Application Scientist.



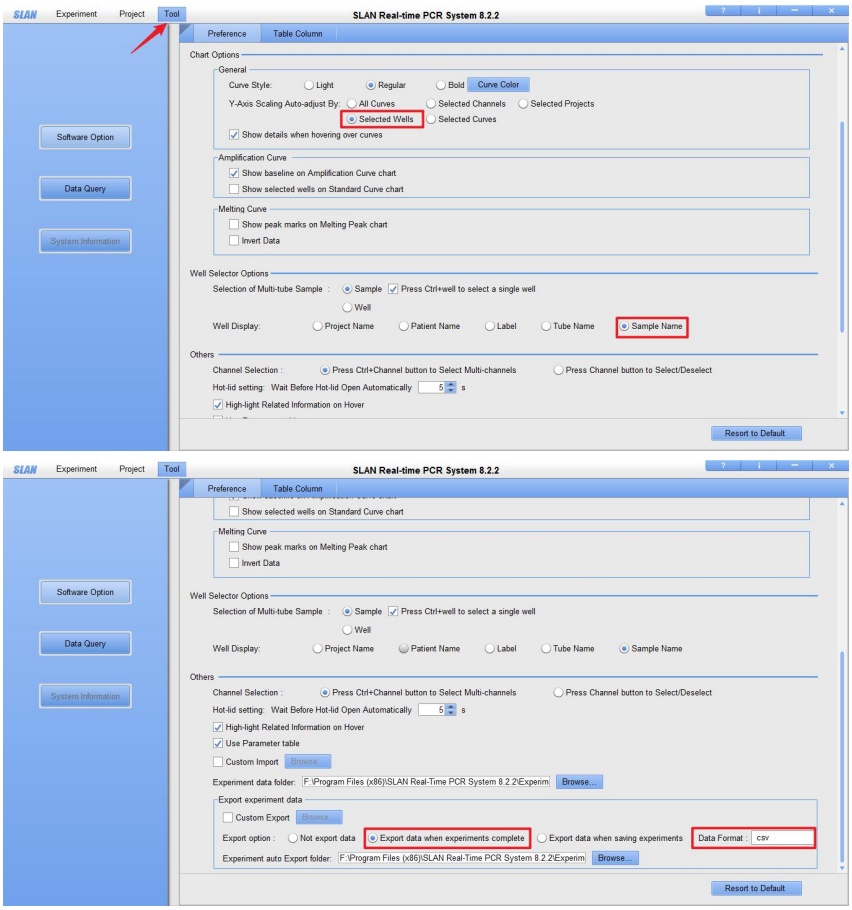


5) Click the “Tool” button in the top bar and set the following parameters:

a) Select “Selected Wells” for “Y-Axis Scaling Auto-adjust By”.

b) Select “Sample Name” for “Well Display”

c) Select “Export data when experiments complete” and set the data format as “csv” for “Export option”.



6) Click “Experiment” button in the top bar.

7) Click “Experiment Wizard” in the left sidebar.

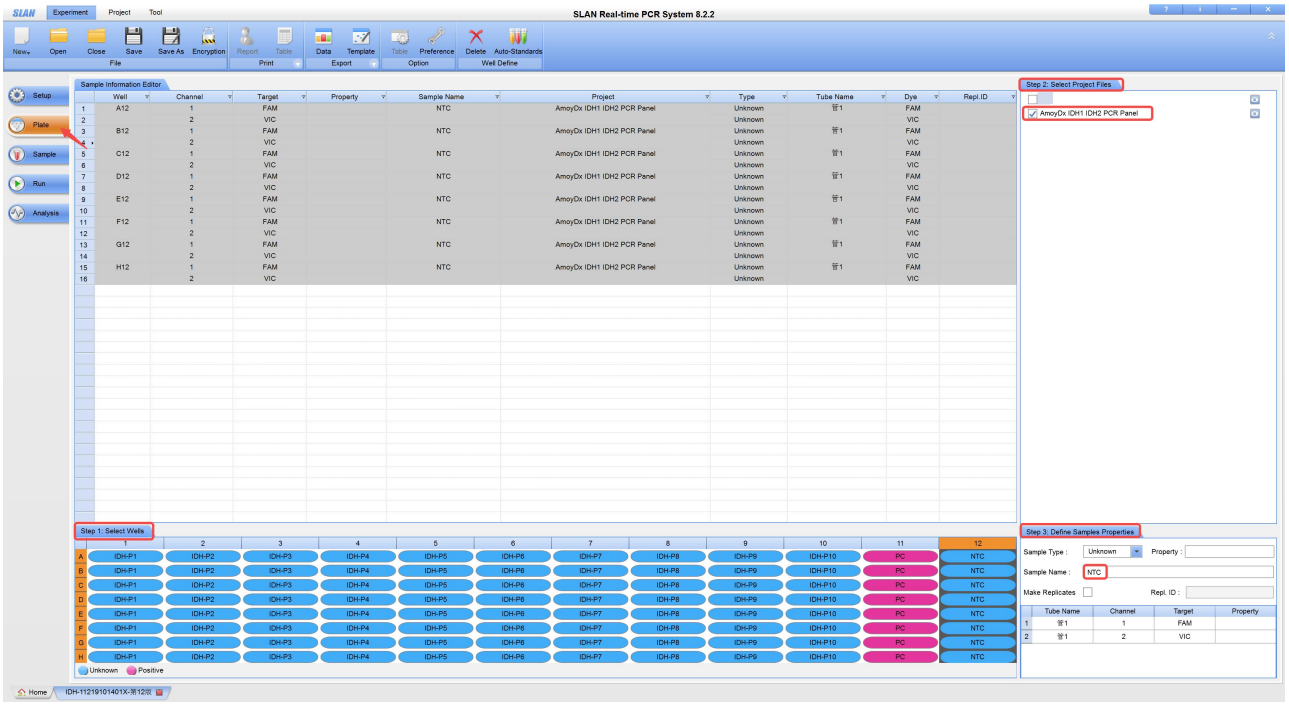
8) Set the experiment properties per your request.

9) Click the “Plate” button on the left sidebar to set the plate information.

a) Highlight the wells you assigned the IDH1/2 PCR Strips.

b) Select the project file “AmoyDx IDH1 IDH2 PCR Panel.prj”.

c) Insert the sample names.



10) Click the “Run” button on the left sidebar and click “Start” to start the PCR process.

4. Result Interpretation

Threshold Setting: For analysis, select a single reaction tube and corresponding fluorescence signal sequentially by tube number (Tubes 1-8), along with the respective Positive Control (PC) and No Template Control (NTC) tubes. Adjust the threshold to 5% of the PC’s peak fluorescence at the 31st cycle to determine the Ct values for each reaction tube.

No Template Control and Positive Control Analysis

1) **NTC (No Template Control):** The FAM signal of tubes 1–7 in the negative control (NTC) should not exhibit a significant amplification curve.

   a) If the FAM signal rises in any of tubes 1–7, the experiment is considered invalid and must be repeated.

   b) If the VIC signal of tubes 1–7 or the FAM signal of tube 8 occasionally rises, but the FAM signal of tubes 1–7 does not show an amplification curve, it does not affect the mutation detection results, and analysis can proceed.

2) **PC (Positive Control):** The FAM and VIC signals of the positive control (PC) should exhibit a significant amplification curve, with a typical Ct value < 20.

Mutation Analysis

1) **External Control (EC):** The FAM signal of reaction tube 8 (external control tube) in the sample should exhibit a significant amplification curve.

   a) For paraffin-embedded samples, the Ct value is recommended to be between 15 and 21; for non-paraffin-embedded samples, the Ct value is recommended to be between 12 and 19. If the Ct value falls within the required range, analysis can proceed.

b) If the Ct value of tube 8 is lower than the range specified in a), it indicates an excessive DNA input. The amount of DNA should be reduced, and the experiment should be repeated. In this case, if the FAM signal of tubes 1–7 does not show a significant amplification curve or the Ct value falls within the negative range, the sample does not need to be re-tested, and the detection result is IDH1/IDH2 gene mutation-negative.

c) If the Ct value of tube 8 is higher than the range specified in a) or if no significant FAM signal amplification is observed, it indicates the presence of PCR inhibitors in the DNA or insufficient DNA input. DNA extraction should be repeated, or the DNA input volume should be increased before re-testing. In this case, if the FAM signal of tubes 1–7 shows a significant amplification curve and falls within the Positive A, the sample does not need to be re-tested, and the detection result is IDH1/IDH2 gene mutation-positive.

2) **Internal Control (IC):** The VIC signal (internal control) in tubes 1–7 of the test sample should exhibit a significant amplification curve. If the internal control does not show a significant amplification curve in tubes 1–7, it indicates the presence of PCR inhibitors in the DNA or insufficient DNA input. DNA extraction should be repeated before re-testing. In this case, if the FAM signal of tubes 1–7 exhibits a significant amplification curve, it is possible that the amplification of the mutation sequence in the reaction tube has inhibited the amplification of the internal control sequence, and analysis can proceed.

3) **DNA Mutation Analysis:** First, determine the mutation Ct values for each reaction tube of the sample, and then determine the external control Ct value for the sample. Since the mutation percentage in the sample varies, the obtained mutation Ct values will also differ. The specific determination criteria are provided in Table 5.

Table 5 Positive and Negative Ct Range

Tube		Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7
Positive A	Mutation Ct	Ct <24	Ct <26	Ct <26	Ct <24	Ct <26	Ct <26	Ct <26
Positive B	Mutation Ct	24≤Ct <26	26≤Ct <28	26≤Ct <28	24≤Ct <26	26≤Ct <28	26≤Ct <28	26≤Ct <28
	ΔCt Cut-off	8	9	9	8	9	9	9
Negative	Mutation Ct	Ct≥26	Ct≥28	Ct≥28	Ct≥26	Ct≥28	Ct≥28	Ct≥28

a) If the Ct values fall within the negative Ct range, the sample is considered negative in that tube or below the detection limit of this kit.

b) If the Ct values fall within the positive A range, the sample is considered positive in that tube.

c) If the Ct values fall within the positive B range, calculate the ΔCt Value:

ΔCt Value = Mutation Ct Value - External Control Ct Value

If the ΔCt value is less than the ΔCt Cut-off value, the sample is considered positive in that tube. Otherwise, it is considered negative.

4) **Cross Reactivity:** Some Positive mutation may cause cross-signals between certain mutation reaction tubes. When a sample shows positive results in two or more reaction tubes (determined according to Table 5), first confirm that the reaction tube with the smallest Ct value represents the true positive mutation. Then, calculate the ΔCt value (Mutation Ct Value - External Control Ct Value) for each reaction tube and determine whether the other reaction tubes exhibit cross-signals based on the cross-signal threshold values listed in

Table 6.

i. If the ΔCt value is smaller than the cross-signal cut-off value, the signal is considered a true positive, and the reaction tube is determined as mutation-positive.

ii. If the ΔCt value is greater than or equal to the cross-signal cut-off value, the signal is considered a cross-signal, and the reaction tube is determined as mutation-negative.

Table 6 Cross-signal cut-off values*

True Positive DNA	Cross-signal Cut-off						
	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7
Tube 1		-	9.13	-	-	-	-
Tube 2	-		-	-	-	-	-
Tube 3	5.06	-		-	-	-	-
Tube 4	-	-	-		5.47	-	-
Tube 5	-	-	-	-		-	-
Tube 6	-	-	-	-	-		5.39
Tube 7	-	-	-	-	-	7.61	

Note:

i. "-" indicates no cross-reactivity.

ii. "*" is based on experimental data from artificially synthesized plasmids.

Automated Analysis:

ARAS is a proprietary software installed on a stand-alone workstation PC developed by AmoyDx intended to be used as a tool to aid in the interpretation of AmoyDx® IDH1/IDH2 PCR Panel. Upon the PCR run completion, import the PCR data into the ARAS, to determine sample's mutation status based on the Ct values of the target mutations.

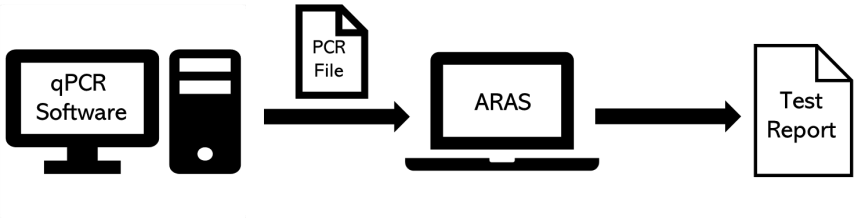


Figure 1 IDH1 IDH2 PCR Data to ARAS Workflow Overview

1) Enter ARAS's IP address 127.0.0.1 in the Chrome browser and provide their account credentials to access ARAS.

2) Click the "Create New Analysis" button and a pop-up window will appear, select the product and instrument you wish to analyze.

3) Click the upload button to select the PCR file to be analyzed and then click "Confirm" to initiate the analysis.

   a) PCR file from SLAN-96S should be in csv format.

4) Assign the sample layout based on the experiment, and then click the "Analysis" button to generate testing results.

5) On the result page, users can verify the accuracy of the test results by reviewing the result list, 96-well plate diagram and the amplified fluorescence curves.

6) On the report page, click the “Generate report” button to generate and download report files for the tested samples.

Note:

- *ARAS is For Research Use Only. Not for use in diagnostic procedures.*
- *PCR file generated from different instruments may necessitate distinct preparation before ARAS analysis. Please adhere the ARAS protocol to ensure that the PCR file is adequately interpretable by ARAS.*
- *For other functions provided by ARAS, please refer to the instructions of ARAS.*

Performance Characteristics

1) The kit's appearance is clean, with clear labeling and no leakage. After thawing, the reagents are clear, with no turbidity or precipitation.

2) Following the instructions, testing was performed on negative corporate reference materials, and the concordance rate for positive reference materials was 100%.

3) Following the instructions, testing was performed on negative corporate reference materials, and the concordance rate for negative reference materials was 100%.

4) There was no cross-reactivity observed with wild-type or other mutation-type sequences that are similar in nucleic acid sequence or homologous, nor with common microorganisms.

5) Under the specified testing conditions, the kit's limit of detection for the covered mutation types of ranges from 1% to 2% at a DNA input of 10 ng per reaction (see Appendix for details).

6) The same negative precision reference material was tested 10 times, with all results negative. The same positive precision reference material was tested 10 times, with all tests detecting positive results.

Note:

- *The above performance characteristics are based on the SLAN-96S platform.*

Limitations

1) This kit is intended for use only by individuals who have received specialized training in PCR techniques.

2) A negative result does not completely exclude the presence of an IDH1 or IDH2 gene mutation. A negative result may occur if the tumor DNA in the sample is too scarce, severely degraded, or if the concentration of the DNA mutation in the amplification reaction system is below the detection limit.

3) Improper sample collection, transportation, handling, as well as incorrect experimental procedures and environmental conditions, may lead to false-negative or false-positive results.

4) This test is limited to the specified sample types and detection systems (including applicable instruments, nucleic acid extraction reagents, and detection methods).

5) The IDH1 IDH2 gene mutation detection range of this reagent only includes the known gene mutation loci explicitly covered by the

detection reagent and does not include gene mutation loci outside the declared range of the kit.

6) The test results obtained with this kit are for research purposes only and should not be used for diagnostic procedures.

Reference

[1] Nassereddine S, Lap CJ, Haroun F, et al. The role of mutant IDH1 and IDH2 inhibitors in the treatment of acute myeloid leukemia. Ann Hematol. 2017;96(12):1983–1991.

[2] Issa GC, DiNardo CD. Acute myeloid leukemia with IDH1 and IDH2 mutations: 2021 treatment algorithm. Blood Cancer J. 2021;11(107).

[3] DiNardo CD, Stein EM, de Botton S, et al. Durable remissions with ivosidenib in IDH1-mutated relapsed or refractory AML. N Engl J Med. 2018;378(25):2386–2398.

[4] Woods A, Norsworthy KJ, Wang X, et al. FDA approval summary: Ivosidenib in combination with azacitidine for treatment of patients with newly diagnosed acute myeloid leukemia with an IDH1 mutation. Clin Cancer Res. 2024;30(7):1226–1231.

[5] James D, Daniel P. Enasidenib for the treatment of acute myeloid leukemia. Expert Rev Clin Pharmacol. 2018;11(7):685–695.

[6] Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med. 2009;360(8):765–773.

[7] Picca A, Berzero G, Di Stefano AL, et al. The clinical use of IDH1 and IDH2 mutations in gliomas. Expert Rev Mol Diagn. 2018;18(12):1041–1051.

[8] Houillier C, Wang X, Kaloshi G, et al. IDH1 or IDH2 mutations predict longer survival and response to temozolomide in low-grade gliomas. Neurology. 2010;75(17):1560–1566.

[9] Louis DN, Perry A, Wesseling P, et al. The 2021 WHO classification of tumors of the central nervous system: A summary. Neuro Oncol. 2021;23(8):1231–1251.

Symbols

	Manufacturer		Catalogue Number
	Batch Code		Use-by Date
	Contains Sufficient for <n> Tests		Temperature Limit
	Consult Instructions For Use		Keep Dry
	This Way Up		Fragile, Handle With Care
	Keep Away from Sunlight		

Appendix

Tube	Target Region	Fluorescence	MUTATION_AA	MUTATION_CDS	COSMIC ID	Name	LoD
1	*IDH1* Exon4	FAM	p.R132S	c.394C>A	28748	IDH1-M1	1%
			p.R132H	c.395G>A	28746	IDH1-M4	1%
		VIC	-	-	-	-	-
2	*IDH1* Exon4	FAM	p.R132C	c.394C>T	28747	IDH1-M2	1%
			p.R132L	c.395G>T	28750	IDH1-M5	1%
		VIC	-	-	-	-	-
3	*IDH1* Exon4	FAM	p.R132G	c.394C>G	28749	IDH1-M3	1%
		VIC	-	-	-	-	-
4	*IDH2* Exon4	FAM	p.R140W	c.418C>T	41877	IDH2-M1	2%
			p.R140G	c.418C>G	1737874	IDH2-M2	2%
			p.R140Q	c.419G>A	41590	IDH2-M3	1%
		VIC	-	-	-	-	-
5	*IDH2* Exon4	FAM	p.R140L	c.419G>T	41875	IDH2-M4	1%
		VIC	-	-	-	-	-
6	*IDH2* Exon4	FAM	p.R172W	c.514A>T	34039	IDH2-M5	1%
			p.R172G	c.514A>G	33731	IDH2-M6	1%
			p.R172M	c.515G>T	33732	IDH2-M8	1%
			p.R172S	c.516G>T	34090	IDH2-M9	1%
		VIC	-	-	-	-	-
7	*IDH2* Exon4	FAM	p.R172K	c.515G>A	33733	IDH2-M7	1%
			p.R172S	c.516G>C	133672	IDH2-M10	1%
		VIC	-	-	-	-	-

Note:

- *The above performance characteristics are based on the SLAN-96S platform. Performance may vary slightly when using other types of instruments.*