

Results Interpretation Guide: Roche LightCycler® 480 Instrument II

Refer to **Section 12** of the Instructions for Use (IFU 0009) for guidance in evaluating test results for run validity, interpretation of patient test results for a single PCR and **Section 13**, and for interpretation of results for patient samples.

1 Run Validity: Control Results

- Record the name of the Test Run in the space "Test Run".
- Analyze PCR run according to *Analysis with the Roche LightCycler 480 Instrument II* in Section 12 of the IFU 0009.
- Record "Well No." and "CP" value or "Undetermined (UD)" for each of the 3 POSITIVE and 3 NEGATIVE Control replicates for Septin9 and ACTB (Internal Control) corresponding with PCR1, PCR2 and PCR3, included in the Test Run. If no CP value is obtained, the Control result is "Undetermined" (meaning no curve was generated).
- Evaluate CP value based TABLE 14: Validity Limits. Check "Pass" or "Fail".
- Confirm if VALID results were obtained for both Positive and Negative Controls before proceeding to patient test results interpretation. Epi proColon POSITIVE and NEGATIVE Controls are considered VALID when all criteria in TABLE 14 are met for all three replicates per Control and both detectors (ACTB and Septin9). If both Controls are VALID, continue to Step 2, Patient Test Results: Interpretation of Results for a Single PCR. **If either or both Controls are INVALID, the data for patient samples processed together with the Controls cannot be interpreted.** Testing must be repeated for all patient samples included in this run.

2 Patient Test Results: Interpretation of Results for a Single PCR

- Record the "Patient Name or ID No." in the space "Patient ID".
- Record "Well No." and "CP" values, or "Undetermined (UD)" for each patient samples for Septin9 and ACTB (Internal Control) corresponding with PCR1, PCR2 and PCR3, included in the Test Run. If no CP value is obtained, the result is "Undetermined" (meaning no curve was generated).
- Evaluate CP value based on TABLE 15: Interpretation of Results for Single PCR, and check appropriate box, INVALID, POSITIVE or NEGATIVE.
- Continue to Step 3, Interpretation of Results for a Patient Sample.

3 Interpretation of Results for a Patient Sample

Refer to Section 13, *Interpretation of Results for a Patient Sample*, TABLE 16: Interpretation of Epi proColon 2.0 CE Test Results, for interpretation of patient test results.

POSITIVE Result: At least 2 of 3 POSITIVE Septin9 PCR replicates

NEGATIVE Result: At least 2 of 3 NEGATIVE Septin9 PCR replicates

INVALID Result: The test is "INVALID" in all other cases

NOTE: When no curve is generated, "Undetermined" is reported. If the Septin9 channel is Undetermined or "UD", that specific well is Septin9 NEGATIVE. If the ACTB channel is Undetermined or "UD", that specific well is NEGATIVE for ACTB and therefore, INVALID.

TABLE 14: Validity Limits of Epi proColon Controls (LightCycler 480 Instrument II)

Result of Control	Determination	Septin9 Result ¹	ACTB Result ²
POSITIVE Control VALID	PCR1	CP* ≤ 40.5	CP* ≤ 30.3
	PCR2	CP* ≤ 40.5	CP* ≤ 30.3
	PCR3	CP* ≤ 40.5	CP* ≤ 30.3
NEGATIVE Control VALID	PCR1		CP* ≤ 37.1
	PCR2	No CP* Provided ("Undetermined")	CP* ≤ 37.1
	PCR3		CP* ≤ 37.1

¹ methylation of Septin9 gene; ² β-actin DNA; Crossing Point

TABLE 15: Interpretation of Results for Single PCR (LightCycler 480 Instrument II)

Single PCR Result	Septin9 Result ¹	ACTB Result ²
Septin9 Positive	CP* < 50	CP* ≤ 33.7
Septin9 Negative	No CP* Provided ("Undetermined")	CP* ≤ 33.7
INVALID	Any Result	CP* ≤ 33.7

¹ methylation of Septin9 gene; ² β-actin DNA; *crossing point

TABLE 16: Interpretation of Epi proColon 2.0 CE Test Results

Test Result	Positive Control Negative Control	Single PCR Results
POSITIVE	VALID	At least two POSITIVE Septin9 PCR
NEGATIVE	VALID	At least two NEGATIVE Septin9 PCR
INVALID	VALID	All other cases
INVALID	INVALID	n/a

The Epi proColon[®] 2.0 CE Test Result Interpretation Forms

For use with the Roche LightCycler[®] 480 Instrument II

There are two forms for recording and interpreting the Epi proColon 2.0 CE test results. Form 1 (below) includes areas for recording Control Results and results for two patients. Every Epi proColon Real-Time PCR test run requires that POSITIVE and NEGATIVE Controls be performed with patient samples. At the top of each Results Form, a space is provided to record the Run Name, Run Date, and the initials of the Technician performing the testing. Form 2 (below) offers space for three additional patients, and this page may be duplicated as needed.

Control results must be VALID before patient results are interpreted; patient results must be POSITIVE or NEGATIVE before being reported; INVALID patient test results should not be reported.

FORM 1

Epi proColon[®] 2.0 CE Results Form LIGHTCYCLER[®] 480 INSTRUMENT II
Not For Use in the United States

Test Run: _____ Operator ID: _____
Run Date: _____

Positive Control					Negative Control						
Well No.	PCR Replicate	Detector	CP	Acceptable Results Criteria	QC Results Pass / Fail	Well No.	PCR Replicate	Detector	CP	Acceptable Results Criteria	QC Results Pass / Fail
PCR1	ACTB			± 30.3 and NOT UD		PCR1	ACTB			± 37.1 and NOT UD	
	Septin9			± 40.5 and NOT UD			Septin9			Result is UD	
PCR2	ACTB			± 30.3 and NOT UD		PCR2	ACTB			± 37.1 and NOT UD	
	Septin9			± 40.5 and NOT UD			Septin9			Result is UD	
PCR3	ACTB			± 30.3 and NOT UD		PCR3	ACTB			± 37.1 and NOT UD	
	Septin9			± 40.5 and NOT UD			Septin9			Result is UD	

Quality Control Run is VALID. Continue with patient test results interpretation. Control Run is INVALID. Do not interpret or report patient test results.

PATIENT ID: _____

Well No.	Determination No.	Detector	CP	Acceptable Results Criteria	Single PCR Result	Final Patient Result
PCR1	ACTB			ACTB > 33.7 or ACTB = UD	<input type="checkbox"/> Invalid	Positive: At least 2 of 3 PCR replicates are positive
	Septin9			ACTB > 33.7 and Septin9 < 50	<input type="checkbox"/> Positive	
PCR2	ACTB			ACTB > 33.7 and Septin9 = UD	<input type="checkbox"/> Negative	Negative: At least 2 of 3 PCR replicates are negative
	Septin9			ACTB > 33.7 or ACTB = UD	<input type="checkbox"/> Invalid	
PCR3	ACTB			ACTB > 33.7 and Septin9 < 50	<input type="checkbox"/> Positive	Invalid: All other cases
	Septin9			ACTB > 33.7 and Septin9 = UD	<input type="checkbox"/> Negative	

Invalid
 Positive
 Negative

PATIENT ID: _____

Well No.	Determination No.	Detector	CP	Acceptable Results Criteria	Single PCR Result	Final Patient Result
PCR1	ACTB			ACTB > 33.7 or ACTB = UD	<input type="checkbox"/> Invalid	Positive: At least 2 of 3 PCR replicates are positive
	Septin9			ACTB > 33.7 and Septin9 < 50	<input type="checkbox"/> Positive	
PCR2	ACTB			ACTB > 33.7 and Septin9 = UD	<input type="checkbox"/> Negative	Negative: At least 2 of 3 PCR replicates are negative
	Septin9			ACTB > 33.7 or ACTB = UD	<input type="checkbox"/> Invalid	
PCR3	ACTB			ACTB > 33.7 and Septin9 < 50	<input type="checkbox"/> Positive	Invalid: All other cases
	Septin9			ACTB > 33.7 and Septin9 = UD	<input type="checkbox"/> Negative	

Invalid
 Positive
 Negative

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PCR3	ACTB			ACTB > 33.7 and Septin9 < 50	<input type="checkbox"/> Positive	Invalid: All other cases
	Septin9			ACTB > 33.7 and Septin9 = UD	<input type="checkbox"/> Negative	

Invalid
 Positive
 Negative

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epi pro colon[®] 2.0 CE

FORM 2

Epi proColon[®] 2.0 CE Results Form LIGHTCYCLER[®] 480 INSTRUMENT II
Not For Use in the United States

Test Run: _____ Operator ID: _____
Run Date: _____

PATIENT ID: _____

Well No.	Determination No.	Detector	CP	Acceptable Results Criteria	Single PCR Result	Final Patient Result
PCR1	ACTB			ACTB > 33.7 or ACTB = UD	<input type="checkbox"/> Invalid	Positive: At least 2 of 3 PCR replicates are positive
	Septin9			ACTB > 33.7 and Septin9 < 50	<input type="checkbox"/> Positive	
PCR2	ACTB			ACTB > 33.7 and Septin9 = UD	<input type="checkbox"/> Negative	Negative: At least 2 of 3 PCR replicates are negative
	Septin9			ACTB > 33.7 or ACTB = UD	<input type="checkbox"/> Invalid	
PCR3	ACTB			ACTB > 33.7 and Septin9 < 50	<input type="checkbox"/> Positive	Invalid: All other cases
	Septin9			ACTB > 33.7 and Septin9 = UD	<input type="checkbox"/> Negative	

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PATIENT ID: _____

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Invalid
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 Negative

PATIENT ID: _____

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	Septin9			ACTB > 33.7 or ACTB = UD	<input type="checkbox"/> Invalid	
PCR3	ACTB			ACTB > 33.7 and Septin9 < 50	<input type="checkbox"/> Positive	Invalid: All other cases
	Septin9			ACTB > 33.7 and Septin9 = UD	<input type="checkbox"/> Negative	

Invalid
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 Negative

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