epi pro colon® 2.0 CE

FOR CLINICAL LABORATORIES
Not For Use in the United States

Results Interpretation Guide: Roche LightCycler® 480 Instrument I

Refer to **Section 11** 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

A. Record the name of the Test Run in the space "Test Run".

B. Analyze PCR run according to *Analysis with the Roche LightCycler 480 Instrument I* in Section 11 of the IFU 0009.

C. 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).

D. Evaluate CP value based on TABLE 11: Validity Limits. Check "Pass" or "Fail".

E. 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 11 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.

TABLE 11: Validity Limits of Epi proColon Controls (LightCycler 480 Instrument I)

Result of Control	Determination	Septin9 Result[1]	ACTB Result[2]
POSITIVE Control VALID	PCR1	CP* ≤ 40.6	CP* ≤ 29.5
	PCR2	CP* ≤ 40.6	CP* ≤ 29.5
	PCR3	CP* ≤ 40.6	CP* ≤ 29.5
NEGATIVE Control VALID	PCR1	No CP* Provided ("Undetermined")	CP* ≤ 36.5
	PCR2		CP* ≤ 36.5
	PCR3		CP* ≤ 36.5

[1] methylation of Septin9 gene; [2] ß-actin DNA; Crossing Point

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

A. Record the "Patient Name or ID No." in the space "Patient ID".

B. 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).

C. Evaluate CP value based on TABLE 12: Interpretation of Results for Single PCR, and check appropriate box, INVALID, POSITIVE or NEGATIVE.

D. Continue to Step 3, Interpretation of Results for a Patient Sample.

TABLE 12: Interpretation of Results for a Single PCR (LightCycler 480 Instrument I)

Single PCR Result	Septin9 Result[1]	ACTB Result[2]
Septin9 Positive	CP* < 50	CP* ≤ 33.1
Septin9 Negative	No CP* Provided ("Undetermined")	CP* ≤ 33.1
INVALID	Any Result	CP* > 33.1 (Or "Undetermined")

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

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.

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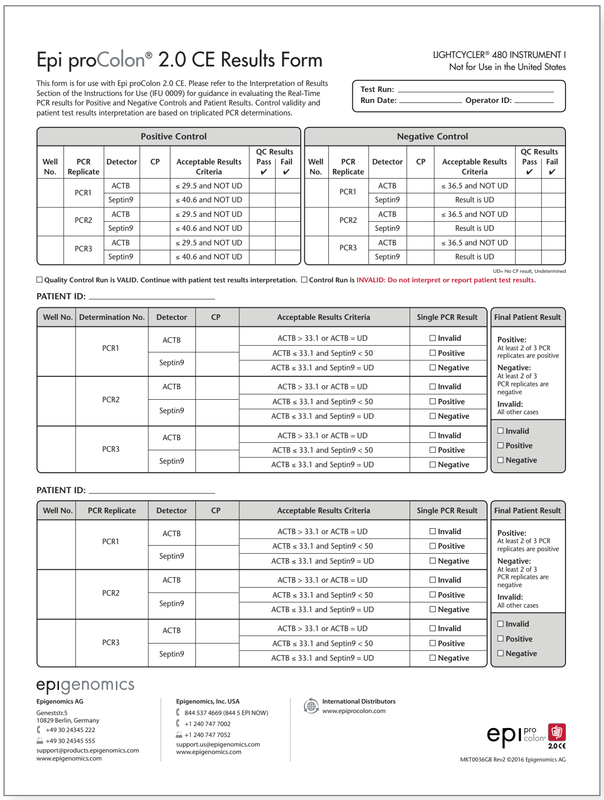
The Epi proColon® 2.0 CE Test Result Interpretation Forms

For use with the LightCycler® 480 Instrument I

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 I
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This form is for use with Epi proColon 2.0 CE. Please refer to the Interpretation of Results Section of the Instructions for Use (IFU 0009) for guidance in evaluating the Real-Time PCR results for Positive and Negative Controls and Patient Results. Control validity and patient test results interpretation are based on triplicated PCR determinations.

Test Run: ____________
Run Date: ________ Operator ID: ________

Positive Control						
Well No.	PCR Replicate	Detector	CP	Acceptable Results Criteria	QC Results Pass ✔	QC Results Fail ✔
	PCR1	ACTB		≤ 29.5 and NOT UD		
		Septin9		≤ 40.6 and NOT UD		
	PCR2	ACTB		≤ 29.5 and NOT UD		
		Septin9		≤ 40.6 and NOT UD		
	PCR3	ACTB		≤ 29.5 and NOT UD		
		Septin9		≤ 40.6 and NOT UD		

Negative Control						
Well No.	PCR Replicate	Detector	CP	Acceptable Results Criteria	QC Results Pass ✔	QC Results Fail ✔
	PCR1	ACTB		≤ 36.5 and NOT UD		
		Septin9		Result is UD		
	PCR2	ACTB		≤ 36.5 and NOT UD		
		Septin9		Result is UD		
	PCR3	ACTB		≤ 36.5 and NOT UD		
		Septin9		Result is UD		

UD= No CP result, Undetermined

☐ 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 > 33.1 or ACTB = UD	☐ Invalid	**Positive:** At least 2 of 3 PCR replicates are positive
		Septin9		ACTB ≤ 33.1 and Septin9 < 50	☐ Positive	**Negative:** At least 2 of 3 PCR replicates are negative
				ACTB ≤ 33.1 and Septin9 = UD	☐ Negative	**Invalid:** All other cases
	PCR2	ACTB		ACTB > 33.1 or ACTB = UD	☐ Invalid	
		Septin9		ACTB ≤ 33.1 and Septin9 < 50	☐ Positive	
				ACTB ≤ 33.1 and Septin9 = UD	☐ Negative	
	PCR3	ACTB		ACTB > 33.1 or ACTB = UD	☐ Invalid	☐ Invalid
		Septin9		ACTB ≤ 33.1 and Septin9 < 50	☐ Positive	☐ Positive
				ACTB ≤ 33.1 and Septin9 = UD	☐ Negative	☐ Negative

PATIENT ID: ____________

Well No.	PCR Replicate	Detector	CP	Acceptable Results Criteria	Single PCR Result	Final Patient Result
	PCR1	ACTB		ACTB > 33.1 or ACTB = UD	☐ Invalid	**Positive:** At least 2 of 3 PCR replicates are positive
		Septin9		ACTB ≤ 33.1 and Septin9 < 50	☐ Positive	**Negative:** At least 2 of 3 PCR replicates are negative
				ACTB ≤ 33.1 and Septin9 = UD	☐ Negative	**Invalid:** All other cases
	PCR2	ACTB		ACTB > 33.1 or ACTB = UD	☐ Invalid	
		Septin9		ACTB ≤ 33.1 and Septin9 < 50	☐ Positive	
				ACTB ≤ 33.1 and Septin9 = UD	☐ Negative	
	PCR3	ACTB		ACTB > 33.1 or ACTB = UD	☐ Invalid	☐ Invalid
		Septin9		ACTB ≤ 33.1 and Septin9 < 50	☐ Positive	☐ Positive
				ACTB ≤ 33.1 and Septin9 = UD	☐ Negative	☐ Negative

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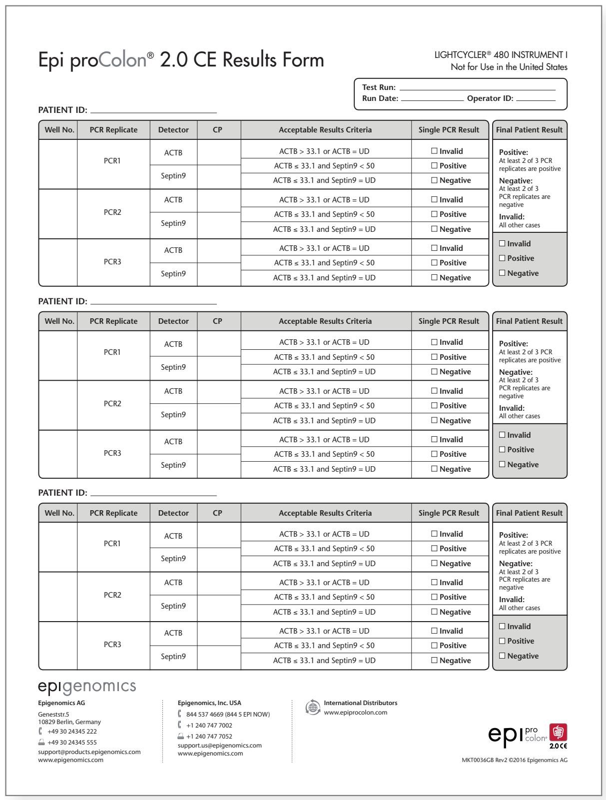
FORM 2



Epi proColon® 2.0 CE Results Form

LIGHTCYCLER® 480 INSTRUMENT I
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Test Run: ____________
Run Date: ________ Operator ID: ________

PATIENT ID: ____________

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				ACTB ≤ 33.1 and Septin9 = UD	☐ Negative	**Invalid:** All other cases
	PCR2	ACTB		ACTB > 33.1 or ACTB = UD	☐ Invalid	
		Septin9		ACTB ≤ 33.1 and Septin9 < 50	☐ Positive	
				ACTB ≤ 33.1 and Septin9 = UD	☐ Negative	
	PCR3	ACTB		ACTB > 33.1 or ACTB = UD	☐ Invalid	☐ Invalid
		Septin9		ACTB ≤ 33.1 and Septin9 < 50	☐ Positive	☐ Positive
				ACTB ≤ 33.1 and Septin9 = UD	☐ Negative	☐ Negative

PATIENT ID: ____________

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				ACTB ≤ 33.1 and Septin9 = UD	☐ Negative	**Invalid:** All other cases
	PCR2	ACTB		ACTB > 33.1 or ACTB = UD	☐ Invalid	
		Septin9		ACTB ≤ 33.1 and Septin9 < 50	☐ Positive	
				ACTB ≤ 33.1 and Septin9 = UD	☐ Negative	
	PCR3	ACTB		ACTB > 33.1 or ACTB = UD	☐ Invalid	☐ Invalid
		Septin9		ACTB ≤ 33.1 and Septin9 < 50	☐ Positive	☐ Positive
				ACTB ≤ 33.1 and Septin9 = UD	☐ Negative	☐ Negative

PATIENT ID: ____________

Well No.	PCR Replicate	Detector	CP	Acceptable Results Criteria	Single PCR Result	Final Patient Result
	PCR1	ACTB		ACTB > 33.1 or ACTB = UD	☐ Invalid	**Positive:** At least 2 of 3 PCR replicates are positive
		Septin9		ACTB ≤ 33.1 and Septin9 < 50	☐ Positive	**Negative:** At least 2 of 3 PCR replicates are negative
				ACTB ≤ 33.1 and Septin9 = UD	☐ Negative	**Invalid:** All other cases
	PCR2	ACTB		ACTB > 33.1 or ACTB = UD	☐ Invalid	
		Septin9		ACTB ≤ 33.1 and Septin9 < 50	☐ Positive	
				ACTB ≤ 33.1 and Septin9 = UD	☐ Negative	
	PCR3	ACTB		ACTB > 33.1 or ACTB = UD	☐ Invalid	☐ Invalid
		Septin9		ACTB ≤ 33.1 and Septin9 < 50	☐ Positive	☐ Positive
				ACTB ≤ 33.1 and Septin9 = UD	☐ Negative	☐ Negative

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