

## Results Interpretation Guide: Applied Biosystems 7500 Fast/Fast Dx Systems

Refer to **Section 10** 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 Applied Biosystems 7500 Fast/Fast DX Systems* in Section 10 of the IFU 0009.
- Record "Well No." and "Ct" 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 Ct value is obtained, the Control result is "Undetermined" (meaning no curve was generated).
- Evaluate Ct value based on TABLE 8: 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 8 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 8: Validity limits of Epi proColon Controls  
(Applied Biosystems 7500 Fast/7500 Fast Dx)

Result of Control	Determination	Septin9 Result <sup>1</sup>	ACTB Result <sup>2</sup>
Positive Control VALID	PCR1	Ct* ≤ 41.1	Ct* ≤ 29.8
	PCR2	Ct* ≤ 41.1	Ct* ≤ 29.8
	PCR3	Ct* ≤ 41.1	Ct* ≤ 29.8
Negative Control VALID	PCR1		Ct* ≤ 37.2
	PCR2	No Ct* Result ("Undetermined")	Ct* ≤ 37.2
	PCR3		Ct* ≤ 37.2

<sup>1</sup> methylation of Septin9 gene; <sup>2</sup> β-actin DNA; \*cycle threshold

TABLE 9: Interpretation of Results for Single PCR

Single PCR Result	Septin9 Result <sup>1</sup>	ACTB Result <sup>2</sup>
Septin9 Positive	Ct* < 45	Ct* ≤ 32.1
Septin9 Negative	No Ct* Result ("Undetermined")	Ct* ≤ 32.1
INVALID	Any Result	Ct* > 32.1 (Or "Undetermined")

<sup>1</sup> methylation of Septin9 gene; <sup>2</sup> β-actin DNA; \*cycle threshold

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

### 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 "Ct" values, or "Undetermined (UD)" for each patient sample for Septin9 and ACTB (Internal Control) corresponding with PCR1, PCR2 and PCR3, included in the Test Run. If no Ct value is obtained, the result is "Undetermined" (meaning no curve was generated).
- Evaluate Ct value based on TABLE 9: 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.

# The Epi proColon® 2.0 CE Test Result Interpretation Forms

## For use with the Applied Biosystem® 7500 FAST/7500 FAST DX

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** APPLIED BIOSYSTEMS 7500 FAST/FAST DX  
Not For Use in the United States

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 replicated PCR determinations.

Test Run: \_\_\_\_\_ Operator ID: \_\_\_\_\_  
Run Date: \_\_\_\_\_

Positive Control					Negative Control								
Well No.	PCR Replicate	Detector	Ct	Acceptable Results Criteria	QC Results Pass ✓	Fail ✗	Well No.	PCR Replicate	Detector	Ct	Acceptable Results Criteria	QC Results Pass ✓	Fail ✗
PCR1	ACTB			± 29.8 and NOT UD			PCR1	ACTB			± 37.2 and NOT UD		
	Septin9			± 41.1 and NOT UD				Septin9				Result is UD	
PCR2	ACTB			± 29.8 and NOT UD			PCR2	ACTB			± 37.2 and NOT UD		
	Septin9			± 41.1 and NOT UD				Septin9				Result is UD	
PCR3	ACTB			± 29.8 and NOT UD			PCR3	ACTB			± 37.2 and NOT UD		
	Septin9			± 41.1 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	Ct	Acceptable Results Criteria	Single PCR Result	Final Patient Result
PCR1	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Positive: At least 2 of 3 PCR replicates are positive
	Septin9			ACTB ± 32.1 and Septin9 < 45	<input type="checkbox"/> Positive	
PCR2	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Negative: At least 2 of 3 PCR replicates are negative
	Septin9			ACTB ± 32.1 and Septin9 < 45	<input type="checkbox"/> Positive	
PCR3	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Invalid: All other cases
	Septin9			ACTB ± 32.1 and Septin9 < 45	<input type="checkbox"/> Positive	

Invalid  
 Positive  
 Negative

PATIENT ID: \_\_\_\_\_

Well No.	PCR Replicate	Detector	Ct	Acceptable Results Criteria	Single PCR Result	Final Patient Result
PCR1	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Positive: At least 2 of 3 PCR replicates are positive
	Septin9			ACTB ± 32.1 and Septin9 < 45	<input type="checkbox"/> Positive	
PCR2	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Negative: At least 2 of 3 PCR replicates are negative
	Septin9			ACTB ± 32.1 and Septin9 < 45	<input type="checkbox"/> Positive	
PCR3	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Invalid: All other cases
	Septin9			ACTB ± 32.1 and Septin9 < 45	<input type="checkbox"/> Positive	

Invalid  
 Positive  
 Negative

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epi pro colon 2.0 CE

FORM 2

**Epi proColon® 2.0 CE Results Form** APPLIED BIOSYSTEMS 7500 FAST/FAST DX  
Not For Use in the United States

Test Run: \_\_\_\_\_ Operator ID: \_\_\_\_\_  
Run Date: \_\_\_\_\_

PATIENT ID: \_\_\_\_\_

Well No.	PCR Replicate	Detector	Ct	Acceptable Results Criteria	Single PCR Result	Final Patient Result
PCR1	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Positive: At least 2 of 3 PCR replicates are positive
	Septin9			ACTB ± 32.1 and Septin9 < 45	<input type="checkbox"/> Positive	
PCR2	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Negative: At least 2 of 3 PCR replicates are negative
	Septin9			ACTB ± 32.1 and Septin9 < 45	<input type="checkbox"/> Positive	
PCR3	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Invalid: All other cases
	Septin9			ACTB ± 32.1 and Septin9 < 45	<input type="checkbox"/> Positive	

Invalid  
 Positive  
 Negative

PATIENT ID: \_\_\_\_\_

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PCR1	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Positive: At least 2 of 3 PCR replicates are positive
	Septin9			ACTB ± 32.1 and Septin9 < 45	<input type="checkbox"/> Positive	
PCR2	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Negative: At least 2 of 3 PCR replicates are negative
	Septin9			ACTB ± 32.1 and Septin9 < 45	<input type="checkbox"/> Positive	
PCR3	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Invalid: All other cases
	Septin9			ACTB ± 32.1 and Septin9 < 45	<input type="checkbox"/> Positive	

Invalid  
 Positive  
 Negative

PATIENT ID: \_\_\_\_\_

Well No.	PCR Replicate	Detector	Ct	Acceptable Results Criteria	Single PCR Result	Final Patient Result
PCR1	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Positive: At least 2 of 3 PCR replicates are positive
	Septin9			ACTB ± 32.1 and Septin9 < 45	<input type="checkbox"/> Positive	
PCR2	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Negative: At least 2 of 3 PCR replicates are negative
	Septin9			ACTB ± 32.1 and Septin9 < 45	<input type="checkbox"/> Positive	
PCR3	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Invalid: All other cases
	Septin9			ACTB ± 32.1 and Septin9 < 45	<input type="checkbox"/> Positive	

Invalid  
 Positive  
 Negative

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