

Results Interpretation Guide: Applied Biosystems 7500 Fast Dx System

Refer to **Section 10** of the Instructions for Use (IFU 0008) for guidance in evaluating test results for validity limits for Epi proColon controls, interpretation of patient test results for a single PCR and for interpretation of results for patient samples. Record results on IFU 0010.

1 Run Validity: Control Results

- Record the name of the Test Run in the space "Test Run".
- Analyze PCR run according to *Assessment of the Validity of the Run by Epi proColon Controls* in Section 10 of the IFU 0008.
- Record "Well No." and "Ct" value or "Undetermined (UD)" for each of the 3 POSITIVE and 3 NEGATIVE Controls for Septin 9 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 of Epi proColon Control Kit. Check "Pass" or "Fail" under QC results on the Results Form IFU 0010.
- 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 3 replicates per Control and both detectors (ACTB and Septin 9). 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 "Ct" values, or "Undetermined (UD)" for each patient sample for Septin 9 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).
- Refer to Section 10, *Assessment of the Validity of a Single PCR of a Patient Specimen* and evaluate Ct values based on TABLE 9: Interpretation of Results for Single PCR, and check appropriate box, INVALID, POSITIVE or NEGATIVE

3 Interpretation of Results for a Patient Sample

Refer to Section 10, *Interpretation of Results for a Patient Sample*, TABLE 10: Interpretation of Epi proColon Test Results, for interpretation of patient test results.

POSITIVE Result: At least 1 of 3 POSITIVE Septin 9 PCR replicates
NEGATIVE Result: All 3 Septin 9 PCR replicates are NEGATIVE
INVALID Result: The test is "INVALID" in all other cases

NOTE: When no curve is generated, "Undetermined" is reported. If the Septin 9 channel is Undetermined or "UD", that specific well is Septin 9 NEGATIVE. If the ACTB channel is Undetermined or "UD", that specific well is NEGATIVE for ACTB and therefore, INVALID.

TABLE 8: Validity Limits of Epi proColon Control Kit

Result of Control	Determination	Septin 9 Result ¹	ACTB Result ²
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

¹ methylation of Septin 9 gene; ² β-actin DNA; *cycle threshold

TABLE 9: Interpretation of Results for Single PCR

Single PCR Result	Septin 9 Result ¹	ACTB Result ²
Septin 9 Positive	Ct* < 45**	Ct* ≤ 32.1
Septin 9 Negative	No Ct* Result ("Undetermined")	Ct* ≤ 32.1
INVALID	Any Result	Ct* > 32.1 Or "Undetermined"

¹ methylation of Septin 9 gene; ² β-actin DNA; *cycle threshold; **Ct values >40 are typically observed for analyte concentrations that are below the Limit of Detection (LoD) of the assay, whereas Ct values <40 are observed for concentrations above the Limit of Detection of the assay.

TABLE 10: Interpretation of Epi proColon Test Results

Test Result	Positive Control Negative Control	Single PCR Results
POSITIVE	VALID	At least one Septin 9 Positive PCR [†]
NEGATIVE	VALID	PCR1: Septin 9 Negative PCR PCR2: Septin 9 Negative PCR PCR3: Septin 9 Negative PCR
INVALID	VALID	All other cases [‡]
INVALID	INVALID	n/a

[†] One single PCR result is Septin 9 Positive; the two remaining single PCR results may have any result (INVALID, Septin 9 Negative, or Septin 9 Positive).

[‡] No single PCR result is Septin 9 Positive; at least one single PCR results is INVALID, the remaining single PCR results may be INVALID or Septin 9 Negative.

The Epi proColon® Results Forms

There are two forms for recording and interpreting the Epi proColon test results. Form 1 (below) includes areas for recording Quality 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 Tech performing the testing (Operator ID). Form 2 (below) offers space for three additional patients and this page may be duplicated as needed.

Quality Control results must be VALID before patient test results are interpreted. Patient test results must be POSITIVE or NEGATIVE before being reported. INVALID patient test results should not be reported and remedial action should be initiated.

IFU 0010 Epi proColon® Results Form APPLIED BIOSYSTEMS 7500 FAST DX

This form is for use with the Epi proColon test. Please refer to the Interpretation of Results section of the Instructions for Use (IFU 0008) 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: _____ 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	<input type="checkbox"/>	<input type="checkbox"/>	PCR1	ACTB			≤ 37.2 and NOT UD	<input type="checkbox"/>	<input type="checkbox"/>
	Septin 9			≤ 41.1 and NOT UD	<input type="checkbox"/>	<input type="checkbox"/>		Septin 9				Result is UD	<input type="checkbox"/>
PCR2	ACTB			≤ 29.8 and NOT UD	<input type="checkbox"/>	<input type="checkbox"/>	PCR2	ACTB			≤ 37.2 and NOT UD	<input type="checkbox"/>	<input type="checkbox"/>
	Septin 9			≤ 41.1 and NOT UD	<input type="checkbox"/>	<input type="checkbox"/>		Septin 9				Result is UD	<input type="checkbox"/>
PCR3	ACTB			≤ 29.8 and NOT UD	<input type="checkbox"/>	<input type="checkbox"/>	PCR3	ACTB			≤ 37.2 and NOT UD	<input type="checkbox"/>	<input type="checkbox"/>
	Septin 9			≤ 41.1 and NOT UD	<input type="checkbox"/>	<input type="checkbox"/>		Septin 9				Result is UD	<input type="checkbox"/>

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.	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 1 of 3 PCR replicates are positive
	Septin 9			ACTB ≤ 32.1 and Septin 9 < 45	<input type="checkbox"/> Positive	
PCR2	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Negative: All 3 PCR replicates are negative
	Septin 9			ACTB ≤ 32.1 and Septin 9 < 45	<input type="checkbox"/> Positive	
PCR3	ACTB			ACTB > 32.1 or ACTB = UD	<input type="checkbox"/> Invalid	Invalid: All other cases
	Septin 9			ACTB ≤ 32.1 and Septin 9 < 45	<input type="checkbox"/> Positive	

Invalid
 Positive
 Negative

PATIENT ID: _____

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PATIENT ID: _____

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epi pro colon
Detecting Cancer In Blood
IFU 0010 Rev 5 ©2016 Epigenomics

Form 1

IFU 0010 Epi proColon® Results Form APPLIED BIOSYSTEMS 7500 FAST DX

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Run Date: _____

PATIENT ID: _____

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PATIENT ID: _____

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epi pro colon
Detecting Cancer In Blood
IFU 0010 Rev 5 ©2016 Epigenomics AG

Form 2

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