

## 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.

### 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 Applied Biosystems 7500 Fast/Fast DX Systems* in Section 10 of the IFU 0009.
- C. 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).
- D.Evaluate Ct value based on TABLE 8: 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 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.

# 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 "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).
- C. Evaluate Ct value based on TABLE 9: 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.

### 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 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>
	PCR1	Ct* ≤ 41.1	Ct* ≤ 29.8
Positive Control VALID	PCR2	Ct* ≤ 41.1	Ct* ≤ 29.8
	PCR3	Ct* ≤ 41.1	Ct* ≤ 29.8
	PCR1		Ct* ≤ 37.2
Negative Control VALID	PCR2	No Ct* Result	Ct* ≤ 37.2
	PCR3	("Undetermined")	Ct* ≤ 37.2

<sup>1</sup> methylation of Septin9 gene; <sup>2</sup> ß-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> ß-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





### 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.

esults for	Positive a	ictions for Use			the interpr	etation of	Results	G	est Run:									Test Run:		
			ontrols and Pa d on triplicate	itient Result	s. Control va	the Real-1 alidity and	lime P patien		un Date:	Ope	rator ID:		=	D:				Run Date:	Opera	ator ID:
		P	Positive Control Negative Control							PCR Replica	e Detector	Ct	Acceptable Results C	Iriteria	Single PCR Result	Final Patie				
					QC R	Results					QC Results				ACTB		ACTB > 32.1 or ACTB	i – UD	Invalid	Positive:
Well No.	PCR Replicate	Detector	Ct Acci	otable Resu Criteria	Its Pass	Fail V	Well No.	PCR Replicate	Detector	Ct Acceptable Criter	teria V V d NOT UD t is UD			PCR1			ACTB ≤ 32.1 and Septi	n9 < 45	Positive	At least 2 i replicates
	PCR1	ACTB	≤ 29.1	and NOT	JD			PCR1	ACTB	≤ 37.2 and M					Septin9		ACTB ≤ 32.1 and Septir	n9 = UD	Negative	Negative
	PCR1	Septin9	≤ 41.1	and NOT	JD			PCRI	Septin9	Result is					ACTB		ACTB > 32.1 or ACTB	I – UD	O Invalid	PCR replic
	PCR2 ACTB	ACTB	≤ 29.1	8 and NOT	JD			PCR2	ACTB	≤ 37.2 and 1				PCR2	-		ACTB ≤ 32.1 and Septi	n9 < 45	Positive	negative Invalid:
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	PCR3	ACTB		.8 and NOT UD			PCR3	ACTB	≤ 37.2 and 1					ACTB		ACTB > 32.1 or ACTB	L= UD	Invalid	□ Invalio	
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Quality	Control R	un is VALID. Co	ntinue with pa	atient test re	sults interpr	etation.	Contr	ol Run is INN	ALID: Do no	t interpret or report pa			Contraction of the second		Septin9		ACTB ≤ 32.1 and Septir		Negative	□Negat
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Well N	. Deterr	nination No.	Detector	Detector Ct		Acceptal	ble Re	ults Criteri	ia	Single PCR Result	Final Patient Result		D:							
			ACTB		ACTRO		12.1 or	ACTB - UD	,	Dinvalid Positive:			PCR Replicate	e Detector	Ct	Acceptable Results C		Single PCR Result	Final Patie	
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			Septin9		ACTB = 32.1 a					□ Negative	replicates are positive Negative:			Septin9		ACTB ≤ 32.1 and Septi		Positive	replicates	
			ACTB			ACTB > 32.1 or ACTB				At least 2 of 3 C Invalid Positive Invalid:		are				ACTB ≤ 32.1 and Septir	n9 = UD	Negative	At least 2	
	PCR2		ACTB	ACID				Septin9 < 4				negative			ACTB		ACTB > 32.1 or ACTB	-	Invalid	PCR replic negative
			Septin9		ACTB ± 32.					Negative		id: ter cases		PCR2	Septin9		ACTB ≤ 32.1 and Septi		Positive	Invalid: All other c
			+	-			ACTB = UD		-		/alid					ACTB ≤ 32.1 and Septir	n9 = UD	□ Negative		
	PCR3		ACTB	ACTB		ACTB > 32 ACTB = 32.				Invalid Positive	Positive Negative			ACTB		ACTB > 32.1 or ACTB	i = UD	Invalid	□ Invalie	
			Septin9		< 0					_			PCR3			ACTB ≤ 32.1 and Septi		Positive	Positi	
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ATIEN	ID:			_										D:						
Well N	. PCR	Replicate	Detector	Ct		Acceptal	ble Re	ults Criteri	ia	Single PCR Result	Final Patie	Patient	Result	PCR Replica	e Detector	Ct	Acceptable Results C	Iriteria	Single PCR Result	Final Pat
			ACTB		ACTB >		32.1 or	ACTB - UD	)	□ Invalid	Porit	Positive:			ACTB		ACTB > 32.1 or ACTB	I = UD	Invalid	Positive:
		PCR1	ACTO				2.1 and Septin9 < 45			Positive	At least 2 of 3 PCR replicates are positive		PCR1	-		ACTB ≤ 32.1 and Septi	n9 < 45	Positive	At least 2 i replicates	
			Septin9			ACTB ≤ 32.1 and Septin9 =				Negative:			Septin9		ACTB ≤ 32.1 and Septir	n9 = UD	Negative	Negativ		
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PCI	PCR2									negative Invalid:			PCR2			ACTB ≤ 32.1 and Septi	n9 < 45	Positive	negative Invalid:	
			Septin9			ACTB = 32.1 and Septin9 < 45 ACTB = 32.1 and Septin9 = UD						Invalid: All other cases			Septin9		ACTB ≤ 32.1 and Septir	n9 = UD	□ Negative	All other o
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