epi pro colon®

Real-Time PCR
Single-Day Test Protocol
Flexible Workflow

Rx Only

epigenomics

Detecting Cancer In Blood.

Intended Use

The Epi proColon test is a qualitative *in vitro* diagnostic test for the detection of methylated Septin 9 DNA in EDTA plasma derived from patient whole blood specimens. Methylation of the target DNA sequence in the promoter region of the *SEPT9_v2* transcript has been associated with the occurrence of colorectal cancer (CRC). The test uses a real-time polymerase chain reaction (PCR) with a fluorescent hydrolysis probe for the methylation specific detection of the Septin 9 DNA target.

The Epi proColon test is indicated to screen adults of either sex, 50 years or older, defined as average risk for CRC, who have been offered and have a history of not completing CRC screening. Tests that are available and recommended in the USPSTF 2008 CRC screening guidelines should be offered and declined prior to offering the Epi proColon test. Patients with a positive Epi proColon test result should be referred for diagnostic colonoscopy. The Epi proColon test results should be used in combination with physician's assessment and individual risk factors in guiding patient management.

Contraindications

The Epi proColon test is not intended to replace colorectal cancer screening tests that are recommended by appropriate guidelines (e.g., 2008 USPSTF guidelines) such as colonoscopy, sigmoidoscopy and high sensitivity fecal occult blood testing.

The Epi proColon test is not intended for patients who are willing and able to undergo routine colorectal cancer screening tests that are recommended by appropriate guidelines.

The Epi proColon test is not intended for patients defined as having elevated risk for developing CRC based on previous history of colorectal polyps, CRC or related cancers, inflammatory bowel disease (IBD), chronic ulcerative colitis (CUC), Crohn's disease, familial adenomatous polyposis (FAP). People at higher risk also include those with a family history of CRC, particularly with two or more first degree relatives with CRC, or one or more first degree relative(s) less than 50 years of age with CRC.

The Epi proColon test has not been evaluated in patients who have been diagnosed with a relevant familial (hereditary) cancer syndrome, such as non-polyposis colorectal cancer (HNPCC or Lynch Syndrome), Peutz-Jeghers Syndrome, MYH-Associated Polyposis (MAP), Gardner's syndrome, Turcot's (or Crail's) syndrome, Cowden's syndrome, Juvenile Polyposis, Cronkhite-Canada syndrome, Neurofibromatosis, or Familial Hyperplastic Polyposis, or in patients with anorectal bleeding, hematochezia, or with known iron deficiency anemia.

Warnings, Precautions and Limitations

The Epi proColon test demonstrated inferiority to a fecal test (OC-Auto® Polymedco, Inc.) for specificity, indicating that the Epi proColon test exhibited a higher rate of false positive results compared to the FIT test. The Epi proColon demonstrated non-inferiority to a fecal test for sensitivity.

A positive Epi proColon test result is not confirmatory evidence for CRC. Patients with a positive Epi proColon test result should be referred for diagnostic colonoscopy.

A negative Epi proColon test result does not guarantee absence of cancer. Patients with a negative Epi proColon test result should be advised to continue participating in a recommended CRC screening program according to screening guidelines.

Screening with Epi proColon in subsequent years following a negative test result should be offered only to patients who after counseling by their healthcare provider, again decline CRC screening methods according to appropriate guidelines. The screening interval for this follow-up has not been established.

The performance of Epi proColon has been established in cross-sectional (i.e., single point in time) studies. Programmatic performance of Epi proColon (i.e., benefits and risks with repeated testing over an established period of time) has not been studied. Performance has not been evaluated for patients who have been previously tested with Epi proColon. Non-inferiority of Epi proColon programmatic sensitivity as compared to other recommended screening methods for CRC has not been established.

The rate of false positive Epi proColon results increases with age. Test results should be interpreted with caution in elderly patients. See Performance Characteristics in Section 13.[1]

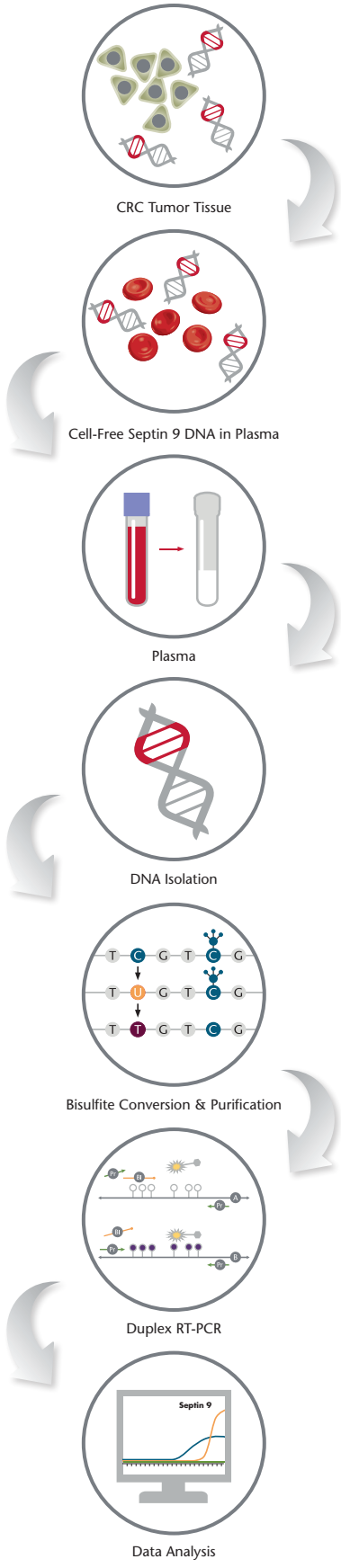
CRC screening guideline recommendations vary for people over the age of 75. The decision to screen people over the age of 75 should be made on an individualized basis in consultation with a healthcare provider.

Positive test results have been observed in healthy subjects and in patients diagnosed with chronic gastritis, lung cancer and in pregnant women.[1,2]

Test results should be interpreted by a healthcare professional. Patients should be advised of the cautions listed in the Epi proColon Patient Guide.

What is Epi proColon®?

Epi proColon is a molecular test that detects methylated Septin 9 DNA in blood. DNA methylation of the *SEPT9* gene is increased in colorectal cancer. Methylated Septin 9 tumor DNA is shed into the bloodstream and displays a unique methylation pattern that is detectable in plasma by Real-Time PCR.[3,4]

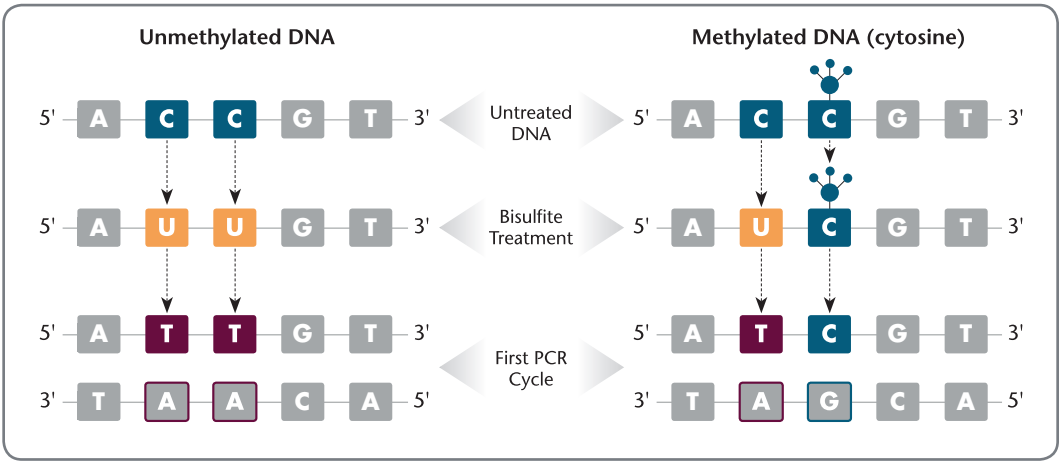


RIGHT:
The Epi proColon test is for use with the Applied Biosystems 7500 Fast Dx Real-Time PCR instrument.

Detecting Methylated Septin 9 DNA

Cytosine residues in the *v2* region of the *SEPT9* gene may become methylated in colorectal cancer tissues. When DNA isolated from plasma samples is treated with a high concentration of bisulfite, unmethylated cytosines are converted to uracil while methylated cytosines remain unchanged (Figure 1). As a consequence of treatment, the DNA sequence is altered based on methylation status and can be analyzed by Real-Time PCR amplification (Figure 1).[3,4]

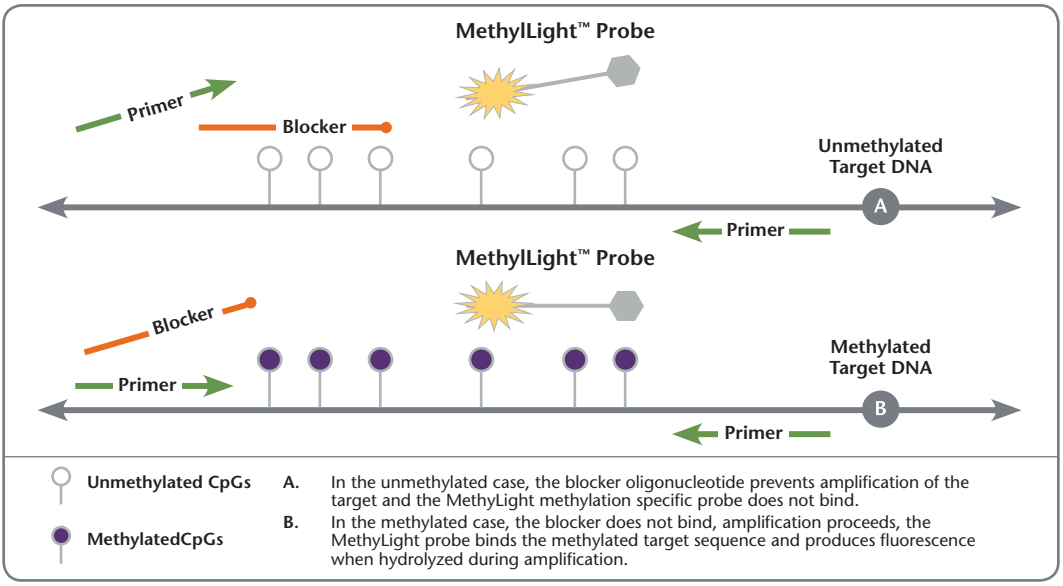
FIGURE 1: Detecting DNA Methylation



HeavyMethyl® Core Technology

The Epigenomics' HeavyMethyl core technology combines the use of primers that amplify the target biomarker regardless of methylation status, with a blocking oligonucleotide to suppress the amplification of unmethylated DNA, and a methylation-specific probe to detect the amplified methylated sequence (Figure 2). The proprietary HeavyMethyl core technology enables detection of low copy number tumor DNA in a background of non-tumor DNA in plasma.[3,4]

FIGURE 2: HeavyMethyl® Real-Time PCR



Epi proColon®
Features & Benefits

Complete Test Kit Offers Convenience and Efficiency

- DNA Extraction and Bisulfite Conversion Reagents
- PCR Reagents
- External Positive and Negative Controls

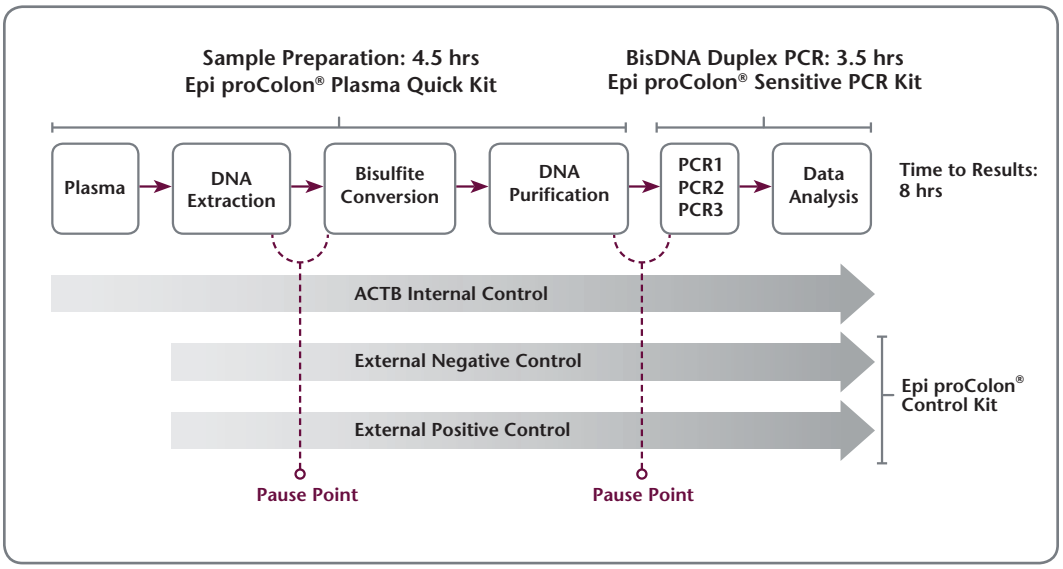
Quality Control Verifies Workflow and Validity

- **Internal Process Control:**
  Co-amplified internal control monitors sample quality, sample preparation and adequate DNA concentration
- **External Controls:**
  Positive and Negative Controls performed identically to patient samples monitor successful workflow and ensure validity of patient test results

Simple Real-Time PCR Test—Basic Molecular Lab Technology

- Familiar PCR technology
- Flexible workflow adapts to staff workload requirements (Figure 3)
- Single day protocol with TTR usually < 8 hours

FIGURE 3: The Epi proColon Test and Workflow



Clinical Trials Overview

Trial One[5]

The first study compared the accuracy of Epi proColon to colonoscopy in 1,544 samples from men and women, 50–85 years of age who were of average-risk for CRC.

Sensitivity (95% CI)	**Specificity** (95% CI)
68.2% (53.4–80.0)	80.0% (77.9–82.1)*
Negative Predictive Value (NPV) (95% CI)	**Positive Predictive Value (PPV) (95% CI)**
99.7% (99.6–99.8)	2.4% (2.0–3.0)

People Not Tested in the Study

- People considered at higher-risk for developing CRC.
- People with rectal bleeding, fresh blood in the stool, or with a known history of iron deficiency.

* Weighted to the Study One Population.

Trial Two[6]

The second study compared the accuracy of Epi proColon to a Fecal Immunochemical Test (FIT) using matched blood and stool samples from 290 people. Epi proColon was found to be statistically non-inferior to FIT with respect to sensitivity but not specificity.

Epi proColon (95% CI) (n=290)	
Sensitivity 72.2% (62.5–80.1) Specificity 80.8% (74.7–85.8)	NPV 99.8% (99.7–99.8) PPV 2.7% (2.0–3.7)

FIT (95% CI) (n=290)	
Sensitivity 68.0% (58.2–76.5) Specificity 97.4% (94.1–98.9)	NPV 99.8% (99.7–99.8) PPV 15.6% (7.2–30.8)

Both Tests

- Identified similar numbers of patients with CRC.
- Identified CRC in all cancer stages and throughout the colon and rectum.

NOTE: Assumes a prevalence of 0.7% based on Study One for Positive and Negative Predictive Values with 95% CI. Predictive values inform how likely disease is given the test result. PPV indicates how likely disease is given a positive test result. NPV indicates how likely absence of disease is given a negative test result.

Trial Three[1]

The third study compared participation in CRC screening among 413 people who were offered either a stool test or a blood test. All people in the study had at least two screening recommendations in the past and were not up-to-date with their screening.

Epi proColon

- 203 people were offered the blood test for CRC screening and 202 completed it (99.5%).
- 30 people had a positive Epi proColon test result; of those, 10 out of the 17 people who completed a colonoscopy had a polyp or adenoma removed.

FIT

- 210 people were offered the stool test for CRC screening and 185 completed it (88.1%).
- 3 people had a positive FIT test result; of those, 1 completed a colonoscopy and had a polyp removed.

Clinical Trials Summary

Trial One[5]

In a large, prospective multicenter clinical trial, 7,941 women and men ages 50 to 85, who were of average-risk for colorectal cancer were enrolled at 32 clinical sites in the US and Germany. The clinical performance of the Epi proColon test was evaluated in 1,544 of the trial participants using colonoscopy as the reference standard (Table 1). The study included all patients with cancer (all stages) or advanced adenomas and a stratified random sample of patients with small polyps, and patients with no evidence of disease (NED), (Tables 2 and 3). The Epi proColon test showed sensitivity, specificity, positive and negative predictive values of 68.2%, 78.8%, 2.4% and 99.7% respectively (Table 1).

TABLE 1: Epi proColon performance with colonoscopy as the reference standard

Sensitivity = 68.2% (95% CI, 53.4–80.0)
Specificity = 78.8% (95% CI, 76.7–80.8)
Specificity* = 80.0% (95% CI, 77.9–82.1)
Negative Predictive Value (NPV) = 99.7% (CI, 99.6–99.8)
Positive Predictive Value (PPV) = 2.4% (CI, 2.0–3.0)
Positive Test Rate = 22.5% (95% CI, 20.6–24.6)

		Epi proColon		
		Negative	Positive	Total
Colonoscopy	CRC	14	30	44
	Non-CRC	1182	318	1500
	Total	1196	348	1544

* Weighted to the Study One population.

PPV = percent probability that a person with a positive test result has CRC
NPV = percent probability that a person with a negative test result does not have CRC

TABLE 2: Epi proColon clinical trial results for different patient groups

Classification	Positives (Total)
NED (No Evidence of Disease)	97 (444)
Polyps	87 (435)
Advanced Adenomas	134 (621)

TABLE 3: Epi proColon clinical trial results for colorectal cancer (CRC) stages

CRC Stage	Epi proColon % (Positives/Total)
Stage I	41.1% (7/17)
Stage II	83.3% (10/12)
Stage III	80.0% (8/10)
Stage IV	100.0% (5/5)
Total Cancers	68.2% (30/44)

Trial Two[6]

The performance of Epi proColon test and a fecal immunochemical test (OC-Auto®) were determined in a multicenter trial at 61 US sites (Table 4). Blood, fecal specimens and clinical data were collected, and performance compared to colonoscopy as the reference method. The study enrolled subjects who: 1) had CRC or a high suspicion of invasive CRC identified by screening colonoscopy—blood and fecal samples were collected at least 10 days after colonoscopy but prior to surgery or intervention; or 2) provided blood and fecal samples prior to bowel prep for screening colonoscopy.

TABLE 4: Performance characteristics for CRC detection of Epi proColon and OC-Auto test

Method	Sensitivity (95% CI)	Specificity (95% CI)
Epi proColon n=301	73.3% (74/101) (63.9–80.9)	81.5% (163/200) (75.5–86.3)
Epi proColon* n=290	72.2% (70/97) (62.5–80.1)	80.8% (156/193) (74.7–85.8)
OC-Auto* n=290	68.0% (66/97) (58.2–76.5)	97.4% (188/193) (94.1–98.9)

* Based on paired samples

NOTE: The observed sensitivity for CRC on paired samples was 4.2% (95% CI, -8.1–16.2) higher for Epi proColon (Table 5). The sensitivity of Epi proColon is statistically non-inferior to the OC-Auto test. For specificity, the difference between the two tests was 16.6% (95% CI, 10.6 –22.9) in favor of the FIT test and does not demonstrate non-inferiority.

Plasma samples were available from 301 eligible people (101 CRC, 29 advanced adenomas (AA), 77 small polyps (SP), 94 with no evidence of disease (NED). Fecal samples were not available from 11 of these subjects (4 CRC, 2 AA, 2 SP and 3 NED). Sensitivity and specificity for the Epi proColon test (73.3%, 81.5%) and OC-Auto tests (68.0%, 97.4%) were in the expected range (Table 4). Based on these results, sensitivity of the Epi proColon test was statistically non-inferior to that of OC-Auto while specificity was not. The Epi proColon test also detected cancers at the earliest clinical stages in this trial (Table 5).

TABLE 5: Epi proColon and OC-Auto clinical trial results for colorectal cancer (CRC) stages

CRC Stage	Epi proColon % (Positives/Total)	FIT % (Positives/Total)
Stage 0	100.0% (2/2)	0.0% (0/2)
Stage I	61.5% (16/26)	65.4% (17/26)
Stage II	80.0% (16/20)	80.0% (16/20)
Stage III	65.2% (15/23)	82.6% (19/23)
Stage IV	92.3% (12/13)	58.3% (7/12)
Unknown	76.5% (13/17)	50.0% (7/14)
Total Cancers	73.3% (74/101)	68.0% (66/97)

TABLE 6: Three-way comparison of Epi proColon, OC-Auto and colonoscopy results

	Diagnostic Accuracy Criteria: Standard Colonoscopy					
	Colorectal Cancer			Non-Colorectal Cancer AA, SP, NED		
	Epi proColon Positive	Epi proColon Negative	Total	Epi proColon Positive	Epi proColon Negative	Total
OC-Auto Positive	50	16	66	1	4	5
OC-Auto Negative	20	11	31	36	152	188
Total	70	27	97	37	156	193

In a three way comparison, the tests were compared with colonoscopy as the reference (Table 7). In this study, combining the two tests would increase sensitivity to 89%, with little impact on specificity.

Trial Three[1]

In a third, prospective multicenter clinical trial (ADMIT), 413 eligible men and women from two health systems were randomized to the Epi proColon blood test or a FIT test (OC-Auto®). All people enrolled in the trial had at least two screening recommendations in the past and were not up-to-date. The primary and secondary study objectives were to compare adherence to CRC screening (Table 7) and compliance to colonoscopy for people who had positive results from either method (Table 8).

Adherence rates for Epi proColon blood test and FIT were 99.5% (95% CI, 97.3–100) and 88.1% (95% CI, 83.0–91.8), respectively, an observed difference in adherence of 11.4% (95% CI, 6.9–15.9). For Epi proColon, 182 of 202 blood samples obtained valid results, leaving 20 blood tests incomplete due to phlebotomy and laboratory errors. For FIT, 179 of 185 stool samples obtained valid results, leaving six samples with no result. Adherence to screening was calculated based on all samples obtained from patients for testing regardless of validity. People with positive results were counseled to undergo colonoscopy. For people with a positive Epi proColon result, 17/30 (56.7%) completed colonoscopy; of these cases, 10/17 (58.8%) resulted in actionable findings (polyps, adenomas) and 0 (0%) were diagnosed as CRC. For people with a positive FIT result, 1/3 (33.3%) completed colonoscopy; of these cases, 1 (100%) resulted in actionable findings (polyps, adenomas) and 0 (0%) were diagnosed with CRC.

TABLE 7: Adherence to CRC Screening with Epi proColon and FIT

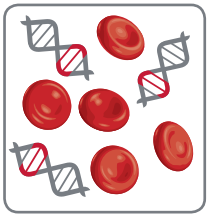
	Epi ProColon			FIT		
	Site 1	Site 2	Total	Site 1	Site 2	Total
Adherent	85	117	202	72	113	185
Non-Adherent	1	0	1	12	13	25
Total	86	117	203	84	126	210

TABLE 8: Adherence to Colonoscopy with Epi proColon and FIT

	Epi ProColon			FIT		
	Site 1	Site 2	Overall	Site 1	Site 2	Overall
Positivity Rate	20.5% (14/68)	14.0% (16/114)	16.5% (30/182)	4.4% (3/68)	0% (0/111)	1.7% (30/179)
Schedule Rate	50.0% (7/14)	81.3% (13/16)	67.7% (20/30)	66.7% (2/3)	N/A	66.7% (2/3)
Compliance Rate	35.7%* (5/14)	75.0%** (12/16)	56.7% (17/30)	33.3% (1/3)	N/A	33.3% (1/3)
Adenoma/Polyp Detection Rate	60.0% (3/5)	58.3% (7/12)	58.8% (10/17)	100% (1/1)	N/A	100% (1/1)

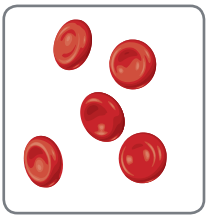
*2 colonoscopies scheduled after study; ** 1 colonoscopy canceled

Understanding Epi proColon Results



A **POSITIVE BLOOD TEST RESULT** indicates that methylated Septin 9 DNA has been detected in the plasma sample tested. Methylated Septin 9 has been associated with the occurrence of colorectal cancer.[3] Because the Epi proColon test is not a confirmatory test for the presence of colorectal cancer, patients with positive Epi proColon test results should be referred for diagnostic colonoscopy.

NOTE: Positive results have been observed in healthy patients and clinically diagnosed patients with chronic gastritis, lung cancer and in pregnant women.[1,2] Because a colonoscopy procedure examines the interior lining of the colon and rectum, CRC is unlikely when no abnormal findings are discovered during this procedure.



A **NEGATIVE BLOOD TEST RESULT** indicates the absence of methylated Septin 9 DNA in the plasma sample tested. Because a negative test result is not confirmatory for the absence of colorectal cancer, people should be advised to continue participating in a colorectal cancer screening program that also includes colonoscopy, fecal tests and/or other recommended screening methods.

NOTE: Studies show that methylated Septin 9 DNA is not present in plasma from all patients with colorectal cancer and therefore, a negative test result does not guarantee absence of cancer.[1] Detection of CRC is dependent on the amount of circulating tumor DNA in the plasma specimen and may be affected by sample collection methods, sample storage, patient factors and tumor stage.[1,3]

Epi proColon Test Kit

30 Patient Plasma Samples • 2 Controls • 96 Well Format

Provided	Required
Epi proColon Plasma Quick Kit (M5-02-001)	BD Vacutainer® K2EDTA Blood Collection Tubes†
Epi proColon PCR Kit (M5-02-002)	
Epi proColon Control Kit (M5-02-003)	

Required Instrumentation
Life Technologies™ Instrument and Software Specification†† Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument with appropriate software version††

† This product has been validated ONLY for use with BD Vacutainer® K2EDTA collection tubes; other reagents and consumables as required for Real-Time PCR are detailed in the Epi proColon Test Kit Instructions for Use (IFU 0008).[1]

†† This product has been validated ONLY for use with the Applied Biosystems 7500 Fast Dx Real-Time PCR instrument and software system. Refer to the Epi proColon Test Instructions For Use (IFU 0008) for description of the appropriate SDS software version, 21 CFR Part 11 Module.

Refer to the Epi proColon Test Instructions for Use (IFU 0008) for more information regarding user requirements.[1]

CPT Code Information

81327, *SEPT9* methylation analysis for CRC.

Find Out More

To learn more about **Epi proColon**, please visit **epiprocolon.com**, and select the "**Q & A**" tab where you will find answers to commonly asked questions.

Email	Support.US@epiprocolon.com
Online	epiprocolon.com
US Toll-free	844 537 4669 (844 5 EPI NOW)
Corporate Phone	240 747 7002
Corporate Fax	240 747 7052

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