DIACARTA

CE IVD XNA Technology

ColoScape™ Colorectal Cancer Mutation Detection Kit

Non-Invasive Testing with Ultra-Sensitivity

HIGHLIGHTS

Non-Invasive Testing
A blood test intended to diagnose colorectal cancer and occurrence at the early and treatable stage

Ultra-Sensitivity
Detect reliably 0.1% to 0.5% Variant Allelic Frequency (VAF) mutant DNA out of wild-type DNA for targeted mutations

Clinical Sensitivity
>60% for advanced adenomas (pre-cancer, stage 0) (compared to 42% for Cologuard®); 95.5% for colorectal cancer (stage I to IV)

Low Input DNA
Minimum 5ng input DNA per reaction. Less than 2 tubes of blood (10mL each) needed for cell-free DNA (cfDNA)

Diagnostic Aid
Aids colonoscopists in the diagnosis of serrated advanced adenomas

Prediction of Therapy Response
Proprietary XNA technology accurately identifies wild-type and mutant status of relevant genes

Fast Result
Less than 4 hours of assay run time

Comprehensive Coverage
Patented gene panel covering 4 genes and 21 mutations

Great Versatility
Validated on the most common qPCR machines with minimized variability: Roche LightCycler® 480, Bio-Rad CFX384 and ABI QuantStudio 5

INTRODUCTION

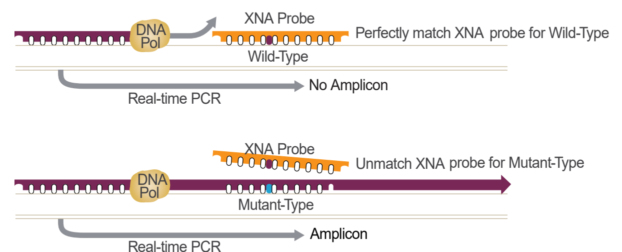
ColoScape™ is a novel multigene mutation biomarker real-time PCR-based assay for qualitative detection of colorectal cancer (CRC) associated somatic mutations in the genes that are frequently mutated in colon cancer patients and are responsible for aberrant colonic epithelial cell proliferation. And it is also intended to improve upon existing CRC detection tests. The assay allows the sensitive detection of the presence or absence of mutations in the targeted regions of the genes interrogated in tissue biopsy (Formalin-Fixed Paraffin-Embedded, FFPE) and plasma samples.

The assay can be performed on readily available qPCR instrumentation that is already present in hospital pathology laboratories. Unlike all of the currently available FDA approved CRC tests on the market, ColoScape™ provides a comprehensive profile of the key CRC 'driver' and 'resistance' mutations (gene variation landscape) and directly informs oncologists of targeted therapy options without the need to reflex to another assay.

POWERED BY XNA TECHNOLOGY

The high sensitivity of this multigene biomarker assay is achieved due to xenonucleic acid (XNA) mediated PCR clamping technology. XNA is a synthetic DNA analog in which the phosphodiester backbone has been replaced by a repeat formed by units of DiaCarta's proprietary novel uncharged backbone chemistry. XNAs hybridize tightly to complementary DNA target sequences only if the sequence is a complete match. Binding of XNA to its target sequence blocks strand elongation by DNA polymerase. When there is a mutation in the target site, and therefore a mismatch, the XNA:DNA duplex is unstable, allowing strand elongation by DNA polymerase. XNA oligomers are not recognized by DNA polymerases and cannot be utilized as primers in subsequent real-time PCR reactions.

XNA Technology Overview



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STREAMLINED WORKFLOW

1. **DNA Isolation & Quantification**
   Extract DNA from plasma using a commercial DNA extraction kit followed by measuring the concentration using fluorometric analysis

2. **Set up qPCR**
   Mix the assay reagents, load into PCR plate, add controls and extracted DNA ~ 30-60 minutes

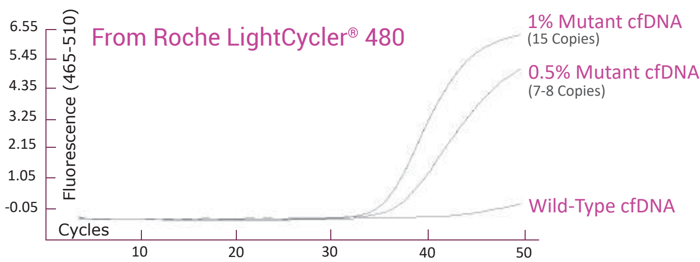
3. **Enter Amplification Parameters**
   Enter amplification parameters on qPCR instrument, load PCR plate and start the run ~ 2.5 hours

4. **Data Analysis**
   Determine the presence or absence of mutations according to the Cq value cutoffs ~ 15 minutes

ANALYTICAL SENSITIVITY

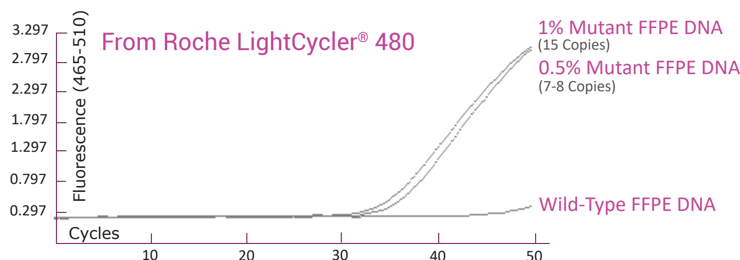
Analytical sensitivity in plasma: Detects as low as 0.5% VAF mutant DNA in a 5ng input.

Amplification curves of ColoScape™ on various concentration of mutant circulating cell-free DNA (cfDNA) from plasma samples are shown in the figure below. Mutation detection limit for the ColoScape™ assay is 0.5% VAF mutant (corresponding to 7-8 copies of mutant DNA) with 5ng total DNA input.



Analytical sensitivity in FFPE: Detects as low as 0.5% VAF mutant DNA in a 5ng input.

Amplification curves of ColoScape™ on various concentration of mutant DNA from FFPE samples are shown in the figure below. Mutation detection limit for the ColoScape™ assay is up to 0.5% VAF mutant (corresponding to 7-8 copies of mutant DNA) with 5 ng total DNA input.



PRODUCT SPECIFICATION

Specification	ColoScape™
Intended Use	For In Vitro Diagnostic Use (CE/IVD) or For Research Use
Sample Type	FFPE and Plasma
Input DNA	5-10ng/Reaction
Pack Size	24 Reactions
Instruments Validated	Roche LightCycler® 480, Bio-Rad CFX384 and ABI QuantStudio 5
Detection Channel	FAM; HEX
Detection Chemistry	TaqMan
Turnaround Time	Less Than 4 hours
Stability	Stable for 12 Months at -25 °C to -15 °C

ORDERING INFORMATION

Product Name	Catalog # (RUO)	Catalog # (CE/IVD)
ColoScape™ Colorectal Cancer Mutation Detection kit	DC-30-0024R	DC-30-0024E

Note: RUO (Research-Use-Only) products are not for use in diagnostic procedures. CE/IVD products are intended for use in diagnostic procedures. To order, please visit www.diacarta.com, email order@diacarta.com or call 1-800-246-8878. To learn more about ColoScape™, please contact information@diacarta.com.

PUBLICATIONS

1. Ultrasensitive detection of unknown colon cancer-initiating mutations using the example of the Adenomatous polyposis coli gene. Gerecke, C., Mascher, C., Gottschalk, U., Kleuser, B., and Scholtka, B., Cancer Prev Res; 2013 6(9): 898-907. DOI: 10.1158/1940-6207.CAPR-13-0145
2. Detection of up to 65% of Precancerous Lesions of the Human Colon and Rectum by Mutation Analysis of APC, K-Ras, B-Raf and CTNNB1. Schneider, M., Scholtka, B., Gottschalk, U., Faiss, S., Schatz, D., Berghof-Jaeger, K., and Steinberg, P., Cancers (Basel); 2011, 3 (1), 91-105. DOI: 10.3390/cancers3010091
3. A gene marker panel covering the Wnt and the Ras-Raf-MEK-MAPK signalling pathways allows to detect gene mutations in 80% of early (UICC I) colon cancer stages in humans. Scholltka, B., Schneider, M., Melcher, R., Katzenberger, Friedrich, D., Berghof-Jaeger, K., Scheppach, W., and Steinberg, P., Cancer Epidemiol., 2009, 33(2), 123-9. DOI: