

DNA Isolation from Formalin-Fixed Paraffin-Embedded Tissue for the Detection of *KRAS* Mutations in Colorectal Cancer

A Method Comparison

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APPLICATION NOTE

Introduction

The *KRAS* protooncogene is one of the most prominent and most commonly mutated RAS family member in colorectal cancer (CRC). Oncogenic mutations of *KRAS* disrupt binding to guanosine triphosphate (GTP) and allow it to remain in an active state (1). The most common mutations in CRC affect codons 12 and 13 (2). Recently, *KRAS* has been shown to be a predictive marker for the response to Epidermal Growth Factor Receptor (EGFR) targeted therapies in patients with metastatic CRC (3). A test strip-based reverse-hybridization assay for the simultaneous detection of 10 mutations in codons 12 and 13 of the *KRAS* gene has been introduced to the market only recently (*KRAS StripAssay*TM; ViennaLab Diagnostics, Vienna, Austria). This assay is based on mutant-enriched PCR using biotinylated primers and reverse-hybridization of amplification products to a test strip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are then detected using streptavidin-alkaline phosphatase and colour substrates. The *KRAS StripAssay*TM assay has a detection limit of 1% mutant sequence in a background of normal DNA and is fully compatible with *KRAS* mutation screening in formalin-fixed paraffin-embedded (FFPE) tissue (4).

This application note aimed at comparing two silica spin column-based DNA preparation kits and an automated magnetic bead-based protocol for the isolation of genomic DNA from FFPE tissue. For this purpose, we isolated genomic DNA from *KRAS*-positive FFPE tissue sections containing varying quota of malignant tissue. Resulting DNA preparations obtained for each of the 3 isolation protocols were then quantified by fluorometry and analyzed using the *KRAS StripAssay*TM.

Materials and methods

Surgically resected tissues obtained from 3 patients operated because of CRC were available. An appropriate paraffin block containing tumor tissue was selected for analysis after reviewing the hematoxylin-eosin (HE) stained slides. The tumor content of each slide was evaluated by an experienced pathologist, and representative sections were then used for mutation analysis with the *KRAS StripAssay*TM. All samples had been previously demonstrated to contain a *KRAS* mutation by DNA sequencing (Table 1).

Table 1: **Characteristics of FFPE sections analyzed in this study.**

FFPE sample	Tumor content (%)	<i>KRAS</i> mutation
A	70	Asp12
B	10	Cys13
C	50	Val12

Genomic DNA from 3 x 5 µm FFPE sections was isolated manually using either the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) or the BiOstic® FFPE Tissue DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA) according to the manufacturers' instructions.

The Prepito Tissue 10 Kit was used with the chemagic Prepito device for automated DNA isolation. Three 5 µm FFPE sections, 300 µl Lysis Buffer and 10 µl Proteinase K solution (20 mg/ml) were incubated overnight at 70°C in a conventional thermoshaker (Eppendorf, Hamburg, Germany). On the next day, samples were centrifuged at 14.000 rpm for 5 minutes and the DNA-containing aqueous phase was carefully removed. Finally, DNA was isolated from 200 µl of the aqueous solution using 150 µl M-PVA Magnetic Beads and 100 µl Elution Buffer.

DNA preparations were quantified using the Quant-iT™ dsDNA HS Assay (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

The *KRAS StripAssay*™ was performed according to the manufacturer's instructions with the DNA template concentration adjusted to 5 ng/µl. It is of note that genomic DNA extracted from FFPE tissue cannot be correctly quantified by UV photometry. Values calculated from optical density (OD) measurements at 260 nm substantially overestimate the DNA template concentration present.

Results

Genomic DNA was isolated from 3 different FFPE tissues either manually using 2 silica spin column-based DNA preparation kits (QIAamp DNA FFPE Tissue Kit; Qiagen and BiOstic® FFPE Tissue DNA Isolation Kit; MO BIO) or essentially automated with the Prepito Tissue 10 Kit (chemagen). DNA preparations were then quantified by fluorometry with DNA yields varying with isolation method and FFPE tissue sample (Table 2). Despite these differences, all samples could be adjusted to a final DNA template concentration of 5 ng/µl considered optimal for mutant-enriched PCR as applied here.

Corresponding *KRAS* mutations were detected in all samples, indicating that each of the 3 isolation protocols was capable of extracting mutant DNA of adequate quantity and quality for use with the *KRAS StripAssay*™ (Fig. 1).

Table 2: **DNA yields obtained from 3 isolation methods tested in this study.**

Method	FFPE sample	DNA concentration in ng/µl	DNA yield in µg/preparation
Prepito Tissue 10 Kit	A	18	1.8
	B	8	0.8
	C	12	1.2
QIAamp DNA FFPE Tissue Kit	A	20	2.0
	B	15	1.5
	C	24	2.4
BiOstic® FFPE Tissue DNA Isolation Kit	A	28	2.8
	B	37	3.7
	C	44	4.4

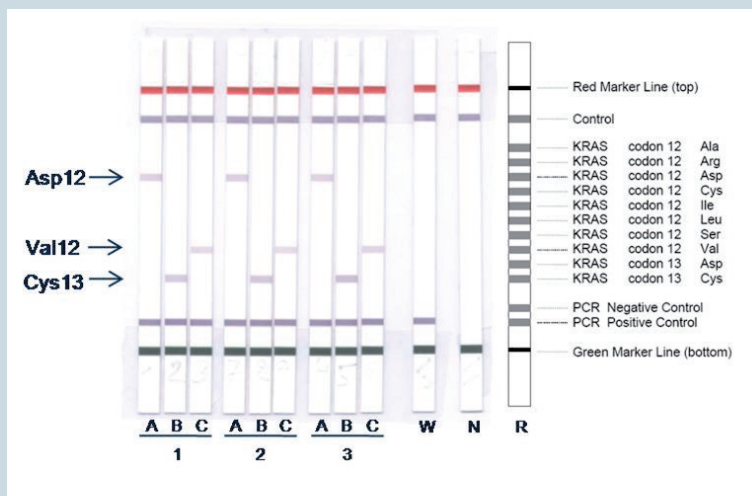


Figure 1:

Identification of mutations using the *KRAS StripAssay™*. Test strips obtained after reverse-hybridization and enzymatic colour development are shown.

FFPE tissue samples: A, B, and C.

DNA isolation by

1: Prepito Tissue 10 Kit

2: QIAamp DNA FFPE Tissue Kit

3: BiOstic® FFPE Tissue DNA Isolation Kit

Controls: W (wild-type control)

N (non template control)

Reference strip: R

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Conclusion

For many years, silica spin column-based protocols have been used in the isolation of DNA from a wide range of tissues and body fluids. Although commercially available, spin column-based approaches involve multiple time-consuming centrifugation steps making automation a difficult task. Automated DNA isolation approaches, however, are time-saving, need less operational input and offer superior reproducibility.

Here we introduced an automated magnetic bead-based protocol (Prepito Tissue 10 Kit; chemagen) to the isolation of genomic DNA from 3 FFPE samples containing defined amounts of malignant tissue. Furthermore, genomic DNA from the same samples were isolated manually using two silica-spin column-based DNA preparation kits (QIAamp DNA FFPE Tissue Kit; Qiagen and BiOstic® FFPE Tissue DNA Isolation Kit; MO BIO). The quality of mutant DNA isolated with the Prepito Tissue 10 Kit was comparable with that obtained from the 2 spin column-based methods as judged by mutant-enriched PCR and reverse-hybridization. The chemagic Prepito facilitates a daily throughput of up to 48 samples, which is difficult to perform manually. For increased throughput (i.e. up to 96 samples), the chemagic Magnetic Separation Module I (chemagen) is available. Both chemagic instruments are based on magnetic separation strategies using chemagen's proprietary M-PVA Magnetic Beads.

Taken together, the Prepito Tissue 10 Kit used with the chemagic Prepito device is a reliable tool for the automated isolation of genomic DNA from FFPE tissue.

References:

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 **Any further question?**

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