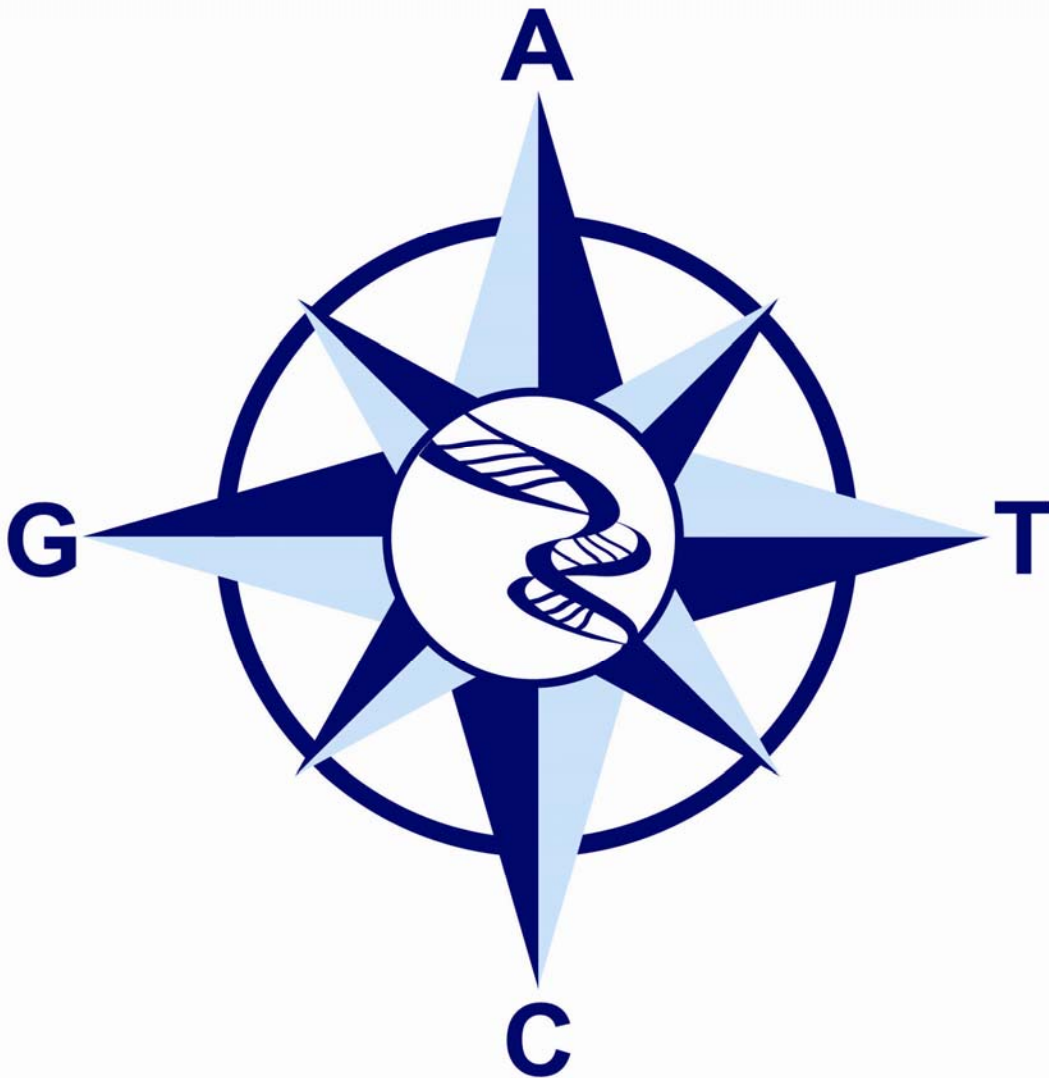




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**Instructions for Use for the
Transgenomic
SURVEYOR® Scan K-RAS
Mutation Detection Kit
CE IVD**



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Manufacturer

SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* is manufactured by Transgenomic, Inc. 12325 Emmet Street, Omaha, NE 68164, USA. Tel 1-402-452-5400. The EC Authorized Representative is Transgenomic Limited, 40 Watt Road, Hillington Park, Glasgow G52 4RY, UK. Tel +44-141-892-8800.

SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD*

This kit is supplied as a single box containing the components indicated below. This User Guide is available as a download.

Intended Use

For professional use only. Transgenomic's SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* is an *in vitro* diagnostic assay that detects mutations in exon 2 of the K-RAS gene. Mutations in codons 12 and 13 are indicated by distinctive assay results. This kit is designed to be used in a clinical diagnostic laboratory by suitably trained personnel testing DNA extracted from formalin-fixed paraffin embedded tissues.

Indications for Use

Clinicians can use the SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* to aid in deciding if patients' colorectal cancer tumours may or may not respond to anti-EGFR (epidermal growth factor receptor) therapeutics such as Vectibix[®] or Erbitux[®].

The SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* **should not** be used for diagnosis of colorectal or any other cancer.

The SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* indicates the presence of mutations in the K-RAS gene exon 2 but does not confirm the sequence identity of the mutation. To confirm the precise mutation detected, further analysis, such as DNA sequencing, would be required.

Results obtained with this kit should be used by a Clinician as an indication of the mutation status of a patient. Other clinical factors should be taken into consideration and the SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* results **should not be used as the sole method used in making decisions regarding any treatment of colorectal cancer patients**. More specifically, samples testing positive for a K-RAS mutation with this kit should be confirmed by DNA sequencing.

Principles of the SURVEYOR Scan K-RAS Mutation Detection Assay

K-RAS

Newly developed therapeutic agents targeting the epidermal growth factor receptor (EGFR), such as cetuximab (Erbitux) and panitumumab (Vectibix), have proven to be effective against colorectal cancer. However, in certain colorectal cancers these drugs are ineffective. Around 40% of colorectal tumors carry K-RAS gene mutations and these mutations have been associated with poor response to EGFR antagonists. K-RAS mutation status can therefore be used to determine whether or not a tumor will respond to anti-EGFR therapy.

The SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* is a diagnostic kit for detecting all sequence and small insertion/deletion mutations in exon 2 of the K-RAS gene. Positive controls are supplied for exon 2 mutations in codons 12 and 13 that have been associated with the lack of effectiveness of anti-EGFR agents.

This kit uses Transgenomic's proprietary SURVEYOR Nuclease and WAVE HS System technologies to give simple and sensitive mutation detection, capable of detecting a mixture of 1% mutant in a

background of 99% non-mutant DNA. Validation studies have demonstrated extremely high concordance with sequencing in well-characterized colorectal cancer samples. Moreover, the resulting SURVEYOR Nuclease digestion patterns for codons 12 and 13 are highly specific. Use of the SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* will both decrease the user's sequencing burden and aid aggressive sequence calling where automated sequencing software fails to resolve the presence of a mutation.

SURVEYOR Nuclease

Transgenomic's SURVEYOR Nuclease is a mismatch-specific plant DNA endonuclease that can scan for known and unknown mutations and polymorphisms in heteroduplex DNA. The enzyme cleaves DNA with high specificity at sites of base-substitution mismatch and other distortions. This DNA endonuclease cuts both strands of a DNA heteroduplex on the 3'-side of the mismatch site¹. Insertion/deletion mismatches and all base-substitution mismatches are recognized, but the efficiency of cleavage varies with the sequence of the mismatch^{1,2}.

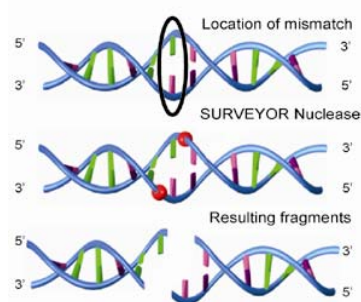


Figure 1. Mode of action of SURVEYOR Nuclease. The endonuclease recognizes a mismatch and cleaves at the 3' side of each base in the mismatch. This cleaves the DNA double stand, leaving a single base 3' overhang.

SURVEYOR Nuclease has been used in a wide range of contexts to detect accurately a variety of mutations and polymorphisms in genes⁵. Notably SURVEYOR Nuclease has been used to verify the presence of known mutations in a number of genes associated with renal cancer³, lung cancer^{4,9,10,12-15}, head & neck cancer⁶, leukemia^{7,16,17}, endometrial cancer⁸ and in radiotherapy outcome prediction¹¹.

The SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* for WAVE HS Systems has been designed to cleave mismatches in K-RAS exon 2 for subsequent analysis by ion-pairing reverse-phase HPLC using the WAVE HS Systems.

Note: Only the DNA Polymerase supplied with this kit should be used for this assay.

Note: Washing procedures specifically recommended and described in this User Guide for the SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* for WAVE HS Systems and the use of DNASep HT Cartridges are different from those used for standard WAVE DHPLC procedures. Please follow the specific recommendations in this manual to maintain optimum performance of your WAVE HS System.

To use this kit successfully, we strongly recommend that you read this manual thoroughly and carefully follow the instructions and guidelines provided. First time users should perform the control experiments outlined in the section "Using K-RAS Control Plasmid DNAs", page 11.

If you have further questions or need assistance, please call **+44 (0) 141 892 8800** (Europe) and ask for "K-RAS support". Alternatively you can e-mail us at:

SURVEYORscan@transgenomic.com

Traceability of Kit Controls

The controls supplied with this kit are plasmid clones of K-RAS exon 2 sequences. All clones have been sequenced to check the fidelity of the sequence by comparison to NCBI Reference Sequence: NG_007524.1.

The K-RAS Control: Wild-Type Exon 2 was constructed by PCR of K-RAS exon 2 from a wild type genomic DNA preparation and cloning.

The K-RAS Positive Control Codon 12 was constructed by site-directed mutagenesis of the K-RAS Control: Wild-Type Exon 2 clone. DNA sequencing confirmed that the only change to the sequence is at codon 12 with a GGT>AGT alteration.

The K-RAS Positive Control Codon 13 was constructed by site-directed mutagenesis of the K-RAS Control: Wild-Type Exon 2 clone. DNA sequencing confirmed that the only change to the sequence is at codon 13 with a GGC>GAC alteration.

See **Using K-RAS Control Plasmid DNAs** on page 11 for more details on DNA sequences of controls.

Components

The SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* is composed of a box with 16 tubes of reagent supplied this kit; there are 4 empty holes in each box.

Catalog Number	Component	100-Reaction Kit (710101-CEIVD) Volume Provided
703310	DNA Polymerase (2.5 U/μL)	100 μL
703315	DNA Polymerase 10X PCR Buffer	1000 μL
703065	dNTPs (10 mM)	500 μL
710151F	K-RAS Primer: Exon 2 Forward (10 μM) (2 tubes)	2 x 250 μL
710151R	K-RAS Primer: Exon 2 Reverse (10 μM) (2 tubes)	2 x 250 μL
710160	SURVEYOR Nuclease W (2 tubes)	2 x 105 μL
710161	SURVEYOR Enhancer W2	105 μL
708049	SURVEYOR Cofactor	105 μL
708027	0.15 M MgCl ₂ Solution	105 μL
708030	Stop Solution	250 μL
710141	K-RAS Control: Wild-Type Exon 2	40 μL
710143	K-RAS Positive Control Codon 12	40 μL
710144	K-RAS Positive Control Codon 13	40 μL
482276	User Guide	Download from web site* http://www.transgenomic.com/pd/surveyor/SurveyorKRAS_CEIVD_ug.asp

Number of Samples that can be Tested with One Kit

The SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* is designed to allow testing of 100 reactions. The total number of samples that can be tested depends upon the average batch size of samples tested at any one time. This is because one set of controls must be tested with samples in each sample batch.

The table below shows the number of samples that can be analyzed with the K-RAS kit depending on the average batch size. This is calculated on the basis that 4 controls are required for every run and a limit of 100 reactions per kit.

When batch size is increased, the number of samples that can be tested in one kit is increased, reducing the average reagent cost per sample.

Batch Size	Number of Controls + Sample amplicons	Tests per Run	Total Runs per Kit	Samples Tested per Kit
1	4 + 1	5	20	20
2	4 + 2	6	16	32
3	4 + 3	7	14	42
4	4 + 4	8	12	48
5	4 + 5	9	11	55
9	4 + 9	13	7	63
16	4 + 16	20	5	80
21	4 + 21	25	4	84
29	4 + 29	33	3	87
46	4 + 46	50	2	92
96	4 + 96	100	1	96

DNA Sequencing

If required, there are sufficient primers supplied for PCR amplification of K-RAS exon 2 to also be used in DNA sequencing of all samples tested.

Additional Required Equipment and Reagents

Additional components and equipment required to use the SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* includes the following:

WAVE System (either 3500HT or 4500HT models), WAVE HSD Accessory, DNASep HT Column (Transgenomic PN DNA-99-3710), WAVE Optimized[®] Buffers (Buffers A, Buffer B and Solution D are Transgenomic PN 553401, 553402 and 553412 respectively; Syringe Wash [for model 3500 systems] and HS Stain solution are PN 553411 and 553442 respectively), 0.2 mL-PCR tubes, 2.0 mL-microcentrifuge tubes, micropipettors, pipette tips, WAVE DNA Sizing Standard (Transgenomic PN 560078), 100-bp DNA mass ladder, molecular biology grade water, ice bath, vortexer, microcentrifuge, thermocycler, agarose gels and agarose gel electrophoresis equipment.

Reagent Preparation

All reagents supplied with this kit are ready to use. Some components will need to be thawed, vortexed or spun in a microcentrifuge before use; check details in Assay Procedure below. Reagents

do need to be combined to produce Master Mixes and reaction mixtures; full details are given in Assay Procedure below – see page 6.

Storage and Shelf Life after First Using Kit

The kit should be stored at between $-18\text{ }^{\circ}\text{C}$ and $-25\text{ }^{\circ}\text{C}$ in a constant temperature freezer until use. Note the Expiry Date of each kit received. Do not use the kit after the Expiry Date has elapsed.

The SURVEYOR Nuclease mixture prepared in Step 7 of SURVEYOR Nuclease Digestion (page 10) should be used immediately as SURVEYOR Nuclease *W* is inactivated over time when in the presence of the other SURVEYOR Nuclease reaction mixture components.

Warnings & Precautions

None of the reagents in this kit present a hazard to health in the quantities supplied. Transgenomic MSD-710101-CEIVD can be downloaded from

<http://www.transgenomic.com/lib/msds/710101.pdf>.

There are no substances in this kit of animal or human origin that present a risk of infection.

This kit should be used only by those persons who have been trained in the appropriate laboratory techniques. When working with the components of this kit, always wear a suitable lab coat, disposable gloves and eye protection. After use the kit components should be disposed of as clinical waste and in accordance with your local rules and regulations.

Aliquots of reagents pipetted from the tubes in this kit are intended for single use only. The components of this kit have been validated as still being stable after 25 freeze-thaw cycles. Do not use this kit if this number of freeze-thaw cycles is exceeded.

Primary Sample Collection, Handling and Storage

The SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* requires DNA extracted from formalin-fixed paraffin embedded colorectal cancer tumor samples. To meet key quality control values for successful use of this kit, the extracted DNA should meet the following standards:

- Q-PCR of the extracted DNA should indicate that there is amplifiable template.
- The 260/280 absorbance ratio should be >1.80 .
- The template concentration for each sample should be $25\text{ ng}/\mu\text{L}$.

Extracted DNA samples not intended for immediate analysis with this kit should be stored at $-20\text{ }^{\circ}\text{C}$.

Assay Procedure

Somatic Mutation Detection with the SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* - An Overview

Mutation detection and confirmation with SURVEYOR Nuclease involves four steps:

Step 1 - Prepare PCR amplicons from mutant (test) and normal (reference) DNA.

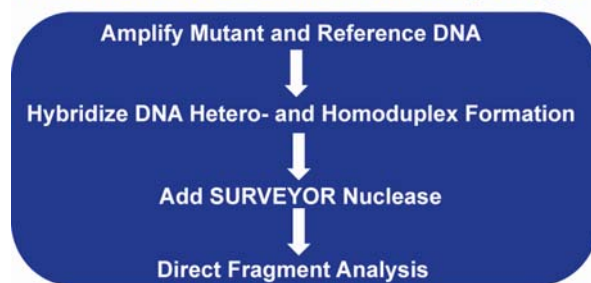
Step 2 – Continuing on from the final PCR amplification cycle the reaction is heated to melt all double strands and then cooled slowly for optimal formation of hetero- and homoduplexes.

Step 3 - Treat the annealed heteroduplex/homoduplex mixture with SURVEYOR Nuclease. The reference DNA alone, treated similarly, serves as a background control.

Step 4 - Analyze the DNA fragments with the WAVE HS System. The formation of new cleavage products, due to the presence of one or more mismatches, is indicated by the

presence of additional peaks. The retention times of the cleavage products indicate the size of the fragments and therefore the location of the mismatch or mismatches.

Mutation Detection in Four Easy Steps



Step-by-Step Instructions

K-RAS Protocol Installation on the WAVE HS System

The K-RAS exon 2 CE protocol for the WAVE HS System can be downloaded into Navigator™ Software for quick and easy set-up. Go to:

<http://www.transgenomic.com/sp/sw/nav/K-RAS%20Protocol/K-RAS%20ProtocolCEIVD.asp>

and follow the instructions for installation of the K-RAS protocol and verification procedures.

WAVE System INITIAL Setup/Cartridge Calibration (Navigator Software) for SURVEYOR Nuclease Applications

Please refer to **Appendix B - WAVE System INITIAL Setup/Cartridge Calibration (Navigator Software) for SURVEYOR Nuclease Applications** in this User Guide.

WAVE System INITIAL Setup/Oven Calibration

Please see WAVE Operator's User Manual for Oven Calibration procedures. **We recommend performing monthly calibrations.**

WAVE HT HS Considerations prior to K-RAS Sample Analysis

1. Prior to running samples, the WAVE DNA Sizing Standard should be run on the K-RAS gradient (**Appendix B – Gradient for K-RAS Exon 2**) to ensure proper functionality of the system.

Template Considerations

1. For FFPE isolated template DNA, use normal laboratory procedures to assess quality and quantity of extracted DNA to ensure there is amplifiable template for PCR.
2. The 260/280 absorbance ratio should be >1.80.
3. To expedite PCR setup, the working template concentration for each sample should be 25 ng/μL. Dilute the template DNA in molecular biology grade water when required.

Primer Considerations

- The sequences of the primers supplied in this kit are as follows:

Amplicon		Sequence
Exon 2	Forward	cggGTTTGTATTA AAAGG TA CTGGTGGAGT
	Reverse	cgggTTTATCTGTATCA AGAATGGT CTCT

NOTE: The primers contain small GC clamps. The 5'-end of exon 2 is very AT rich.

- The amplicon sequences are as follows:
 - Forward primers are highlighted in **Green**. Reverse primers are highlighted in **Red**. Coding regions are highlighted in **Gray**. The most common mutation regions are highlighted in **Purple**. Capitalized letters that are not highlighted are non-coding cDNA regions.

K-RAS Control: Wild Type Exon 2

MD Loci: 10428 - 10706

Size: 286 bp

cgggTTTGTATTAAAAGG**TA**CTGGTGGAGTATTGATAGTGATTAACCTATGTGTGACATGTTCTAATAGTCACATTTTCATTTTT
 attataag**GCCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTGGCCGTA**
GGCAAGAGTGCCTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATC
CAACAATAGAGgtaaatctgttttaaatatgcatattactggtgca**aggaccattctttgatacagataaa**cccg

Amplification Protocol

- The Transgenomic premixed dNTP solution (PN 703065) is supplied at a working concentration of 10 mM total deoxynucleotide (2.5 mM of each of the four deoxynucleotides).
- The forward and reverse primers (PN 710151F and 710151R, respectively) for each amplicon are supplied at 10 µM.
- Remove 10 µM primers, 10 mM premixed dNTP solution and DNA Polymerase 10X PCR Buffer (PN 703315) from the freezer and thaw on ice.
- Prepare Master Mix on ice.
- Use the following table as a guide for preparing the Master Mix for K-RAS exon 2:

Number of Reactions:	1.00
Volume Calculation:	
Volume of Water (µL)	33.0**
DNA Polymerase 10X PCR Buffer (µL)	5.0
dNTPs (µL)	4.0
K-RAS Primer: Exon 2 Forward (µL)	2.5
K-RAS Primer: Exon 2 Reverse (µL)	2.5
DNA Polymerase (µL)	1.0
Total Volume Master Mix:	48.0
Volume of extracted DNA to add (µL at 25 ng/ µL)	2.0**
Total Volume PCR Reaction:	50.0

**Note: for extracted DNA concentrations of <25 ng/µL, increase the volume of extracted DNA proportionately and also decrease the volume of water in the Master Mix by this same amount to result in 50 µL per reaction. All samples prepared with this master mix will need to have

Instructions For Use of the SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD*

extracted DNA diluted to approximately the same lowest concentration level. Using extracted DNA concentrations of <5 ng/μL is not recommended.

- Calculate required volumes for Master Mix by multiplying volumes shown in above chart with the total number of samples to be analyzed plus 4 additional reactions for the controls.
- Label 0.2 mL-PCR tubes or wells of a 96-well plate with appropriate sample information.
- Label a 2.0 mL-centrifuge tubes for Master Mix preparation.
- Add required volume of molecular biology grade water to the 2.0 mL-centrifuge tube labeled Master Mix.
- Vortex DNA Polymerase 10X PCR Buffer for ~ 10 s.
- Add required amount of DNA Polymerase 10X PCR Buffer to the 2.0 mL-centrifuge tube.
- Vortex 10.0 mM premixed dNTP working solution for ~ 10 s.
- Add required volume of the 10.0 mM premixed dNTPs working solution to the 2.0 mL-centrifuge tube.
- Add required volume of the K-RAS Primer: Exon 2 Forward to the 2.0 mL-centrifuge tube.
- Add required volume of the K-RAS Primer: Exon 2 Reverse to the 2.0 mL-centrifuge tube.
- Take DNA Polymerase (PN 703310) out of the freezer.
- Centrifuge DNA Polymerase for ~10 s.
- Vortex the DNA Polymerase for ~10 s.
- Add required volume of DNA Polymerase to the 2.0 mL-centrifuge tube.
- Cap the 2.0 mL-centrifuge tube containing Master Mix.
- Vortex the 2.0 mL-centrifuge tube for ~30 s before use.
- Store on ice until use.
- Pipet 48.0 μL of Master Mix into appropriate wells, changing pipet tips in between if using a single channel pipet. If using a repeat pipettor, ensure that there is no spillage/splashing from well to well. Keep plate on ice.
- To the appropriate wells add 2.0 μL of each sample template DNA, each control template DNA (PN 710140, 710143, 710144) or template free control (water).
- Once pipetting is done, cap each well with the 8-cap strips (if using a 96-well plate) or cap the 0.2 mL-PCR tubes. Make sure the caps are sealed securely.
- Vortex (~1/2 speed) for 30 s.
- Inspect the plates or 0.2 mL-PCR tubes. Verify that the solution is at the bottom of the well. If not, centrifuge. After centrifuging, reduce the speed of the vortexer and mix for ~ 15 s.

Thermal Cycler Program for Amplification Protocol

- Use the following thermocycler protocol for PCR Amplification and heteroduplex formation:

Initial denaturing	95°C	5 min
15 cycles touchdown	95°C	30 sec
	62°C, -0.5°C/cycle	30 sec
	72°C	25 sec
30 cycles amplification	95°C	30 sec
	55°C	30 sec
	72°C	25 sec
Final extension	72°C	2 min
Heteroduplex formation	95°C	2 min
	4°C	Hold

Quality Control of PCR Products

1. Amplicon quality and quantity should be checked by gel electrophoresis or WAVE DHPLC before proceeding to SURVEYOR Nuclease digestion.
2. Analyze an aliquot of the PCR product along with several different amounts of a 100-bp DNA mass ladder.
3. Use the ladder to estimate the concentration of the amplified DNA.
4. Only a single band >20 ng/μL corresponding to the main product should be observed.
5. If multiple bands are present, ensure quality of input template DNA was sufficient (see **Appendix A – Troubleshooting Guide**).
6. If no product is observed, ensure quality of input template DNA was sufficient (see **Appendix A – Troubleshooting Guide**). If quality meets specifications increase the volume of template to 4.0 μL per 50 μL reaction (reduce water per reaction to 31.0 μL).
7. No PCR products should be visible in the no template control sample. If DNA products are visible, with this control contamination is likely; see Troubleshooting Guide page 20.

SURVEYOR Nuclease Digestion

1. After the sample PCR is deemed of sufficient quality and quantity, perform the SURVEYOR Nuclease digestion reaction as described below.
2. Thaw the 0.15 M MgCl₂ Solution and SURVEYOR Enhancer Cofactor on ice..
3. Add 10.0 μL of each sample to a new 0.2 mL-PCR tube or well of a 96-well plate.
4. If doing multiple samples, prepare a fresh mixture of 0.15 M MgCl₂ Solution, SURVEYOR Enhancer Cofactor, SURVEYOR Enhancer W2 and SURVEYOR Nuclease W (SURVEYOR Nuclease mixture).
 - a. Centrifuge each reagent before use.
 - b. Gently vortex each before pipetting.
 - c. For each digestion, add the following components to a 0.2 mL-PCR (or larger) microcentrifuge tube.
 - 1.0 μL 0.15 M MgCl₂ Solution (PN 708027)
 - 1.0 μL SURVEYOR Enhancer Cofactor (PN 708049)
 - 1.0 μL SURVEYOR Enhancer W2 (PN 710161)
 - 2.0 μL SURVEYOR Nuclease W (PN 710160)
5. Centrifuge the SURVEYOR Nuclease mixture for 10 s on low speed.
6. Gently vortex SURVEYOR Nuclease mixture for 10 s on low speed.
7. Place SURVEYOR Nuclease mixture on ice until use.
8. Pipet 5.0 μL-aliquot of the SURVEYOR Nuclease mixture to each 10.0 μL hybridized PCR product.
9. When pipetting is completed, centrifuge the digestion 0.2 mL-PCR tubes or 96-well plate for 10 s.
10. Gently vortex the sample 0.2 mL-PCR tubes or 96-well plate for 10 s.
11. Incubate at 42 °C for **30** min.
12. Add 1.0 μL Stop Solution (PN 708030) to each tube or well and vortex gently (total SURVEYOR Nuclease reaction volume is 16.0 μL).
13. Inject 8.0 μL of each digestion using the gradients specified. Please refer to **Analysis of K-RAS Exon 2 using SURVEYOR Nuclease** page 12.

Note: The SURVEYOR Nuclease mixture prepared in Step 7 should be used immediately as SURVEYOR Nuclease W is inactivated over time when in the presence of the other SURVEYOR Nuclease reaction mixture components.

Workflow Considerations

The kit is designed to allow mutation analysis of 100 samples; Figure 11 shows a 96-well plate layout with controls and 92 samples. Smaller batches of samples can be run, but the controls and WAVE DNA Sizing Standard should still be run every time. There are sufficient control materials in the kit for all combinations of sample batch sizes to be used.

In general, processing of samples should be carried out from start to finish as described in this User Guide. If processing of a sample is stopped before completion of all steps, the DNA should be stored at -20 °C until the next step is carried out. However, exposure of any frozen sample to repeated freeze-thaw cycles should be avoided and storage at -20 °C of PCR amplified DNA or SURVEYOR Nuclease digestion products for extended periods (>1 week) should be avoided.

Control Procedures

Quality Control of the SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD*

Control Plasmid DNAs are included in the kit to provide quality control checks at specific steps in the assay procedure. For the Amplification Protocol (page 8), these controls provide a means to ensure the Master Mix is correctly prepared and amplification is functioning properly. No template controls (where water is added in place of template DNA) are also recommended to check for possible contamination of kit components with a DNA template.

At the SURVEYOR Nuclease digestion stage, the amplicons from these 3 Control Plasmid DNAs provide an effective check that the cleavage reaction conditions (SURVEYOR Nuclease mixture preparation and incubation conditions) were satisfactory. At the analysis stage, the WAVE System traces of these SURVEYOR Nuclease digested control amplicons provide guidance of where the codon 12 and 13 mutations, even at low levels, will elute (see Figures 3 and 4).

If either the PCR amplicons or the SURVEYOR Nuclease cleavage fragments derived from the Control Plasmid DNAs do not match the results outlined in Quality Control of PCR Products (page 10) or Examples of Results (page 13), consult the Troubleshooting Guide in Appendix A or contact Transgenomic Technical Support before proceeding with further steps in the analysis of patient samples.

Using K-RAS Control Plasmid DNAs

The kit is supplied with three control DNAs:

K-RAS Control: Wild-Type Exon 2; PN 710141

K-RAS Positive Control Codon 12; PN 710143

K-RAS Positive Control Codon 13 ; PN 710144

These control DNAs are plasmids with inserts. The Positive Controls each contain two plasmids: a 50:50 mix of the Wild-Type Control and a mutation clone differing from the Wild-Type at a single base pair. The controls are provided in separate vials, each at a concentration of 2.5 ng/μL.

Forward and reverse primers needed for PCR amplification are supplied separately in the kit. The sequence of the Wild-Type and Positive Controls are shown below.

Forward primers are highlighted in **Green**. Reverse primers are highlighted in **Red**. Coding regions are highlighted in **Gray**. The most sequence differences in the Positive Controls are highlighted in **Purple**. Capitalized letters that are not highlighted are non-coding cDNA regions.

K-RAS Control: Wild Type Exon 2

MD Loci: 10428 - 10706

Size: 286 bp

cgggtttgtattaaaaggctactggaggagatttgatagtgattaaccttatgttgacatgttctaataatagtcacatttcattat
attataagGCCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTGGCGTA
GGCAAGAGTGCCTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATC
CAACAATAGAGgtaaactctgttttaaatgcatattactgggc**aggaccattcttfgatacagataaa**cccg

K-RAS Positive Control Codon 12

MD Loci: 10428 - 10706

Size: 286 bp

cgggtttgtattaaaaggctactggaggagatttgatagtgattaaccttatgttgacatgttctaataatagtcacatttcattat
attataagGCCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGAGCT[G/A]GTGGCG
TAGGCAAGAGTGCCTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGAT
CCAACAATAGAGgtaaactctgttttaaatgcatattactgggc**aggaccattcttfgatacagataaa**cccg

K-RAS Positive Control Codon 13

MD Loci: 10428 - 10706

Size: 286 bp

cgggtttgtattaaaaggctactggaggagatttgatagtgattaaccttatgttgacatgttctaataatagtcacatttcattat
attataagGCCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTG[G/A]CG
TAGGCAAGAGTGCCTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGAT
CCAACAATAGAGgtaaactctgttttaaatgcatattactgggc**aggaccattcttfgatacagataaa**cccg

Please follow instructions found in Amplification Protocol (page 8), SURVEYOR Nuclease Digestion (page 10) and Analysis of K-RAS Exon 2 using SURVEYOR Nuclease (page 12) for use of these controls.

WE STRONGLY RECOMMEND THAT FIRST TIME USERS PERFORM EXPERIMENTS WITH THE CONTROLS ALONE BEFORE TESTING GENOMIC SAMPLES

Interpretation of Results

Analysis of K-RAS Exon 2 using SURVEYOR Nuclease

The K-RAS exon 2 CE Protocol Template for the WAVE HS System can be downloaded into Navigator Software for quick and easy set-up. Go to

<http://www.transgenomic.com/sp/sw/nav/K-RAS%20Protocol/K-RAS%20ProtocolCEIVD.asp>

and follow the instructions for installation of the K-RAS protocol and verification procedures.

1. For manual set-up of the WAVE HS System see the parameters for gradient prediction in **Appendix B: WAVE HS System Parameters for K-RAS Protocol**.

Note: we recommend that the installed protocol is used in preference to manual set-up.

2. Please note that, for comparison/control purposes, always perform SURVEYOR Nuclease digestion on both the controls (wild type and positive controls) and the sample DNAs and run in the same WAVE System tray.
3. In addition, the Transgenomic Size Standard (PN 560078) can be run prior to sample analysis to ensure that the WAVE System is operating properly. A blank run (0 µL injection) should be

run using the K-RAS Exon 2 Gradient. Then, 8 μ L of the WAVE DNA Sizing Standard should be injected on the K-RAS Exon 2 Gradient – see **Figure 2** for expected results.

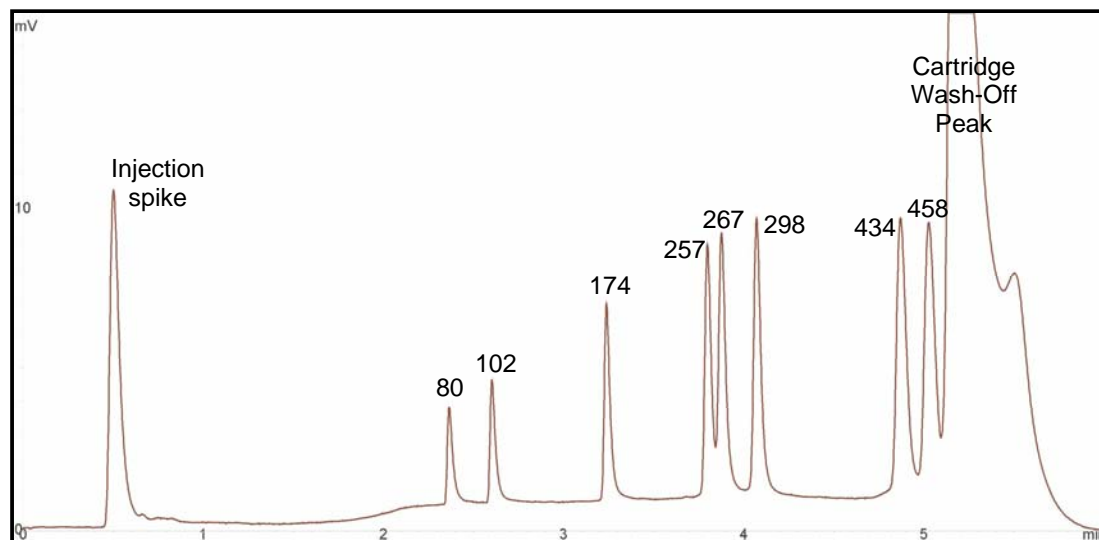


Figure 2 WAVE DNA Sizing Standard injection using K-RAS exon 2 gradient (UV detection). Fragments sizes are indicated in base-pairs.

For details of gradient for K-RAS Exon 2 please refer to **Appendix B – Gradient for K-RAS Exon 2**.

Examples of Results

Examples of results obtained using the SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* for WAVE HS Systems are shown in Figures 3 and 4 below. In these examples, the process outlined in the Overview section was followed precisely.

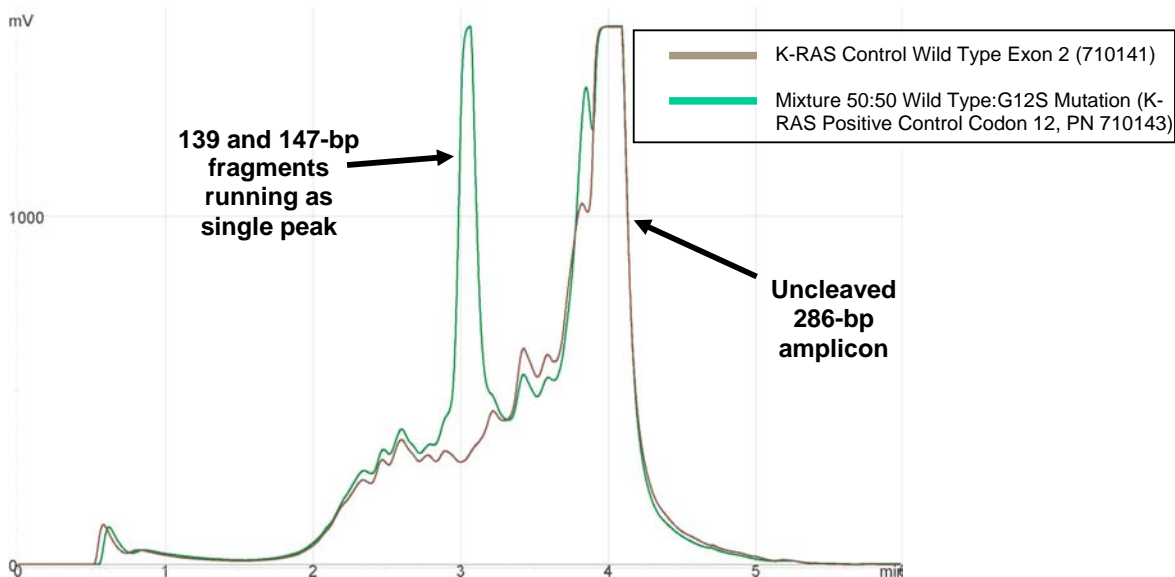


Figure 3 shows SURVEYOR Nuclease digestion products from the 286-bp exon 2 codon 12 amplicon heteroduplexes elute as a single peak. The templates used in this PCR were the K-RAS Control: Wild-Type Exon 2 and K-RAS Positive Control Codon 12. This G12S mutation produces G-T and C-A heteroduplexes which yield 139 & 147-bp fragments after SURVEYOR Nuclease digestion; these fragments' peaks are not resolved on this gradient and run as a single peak. Digestion products were analyzed on a WAVE HS system.

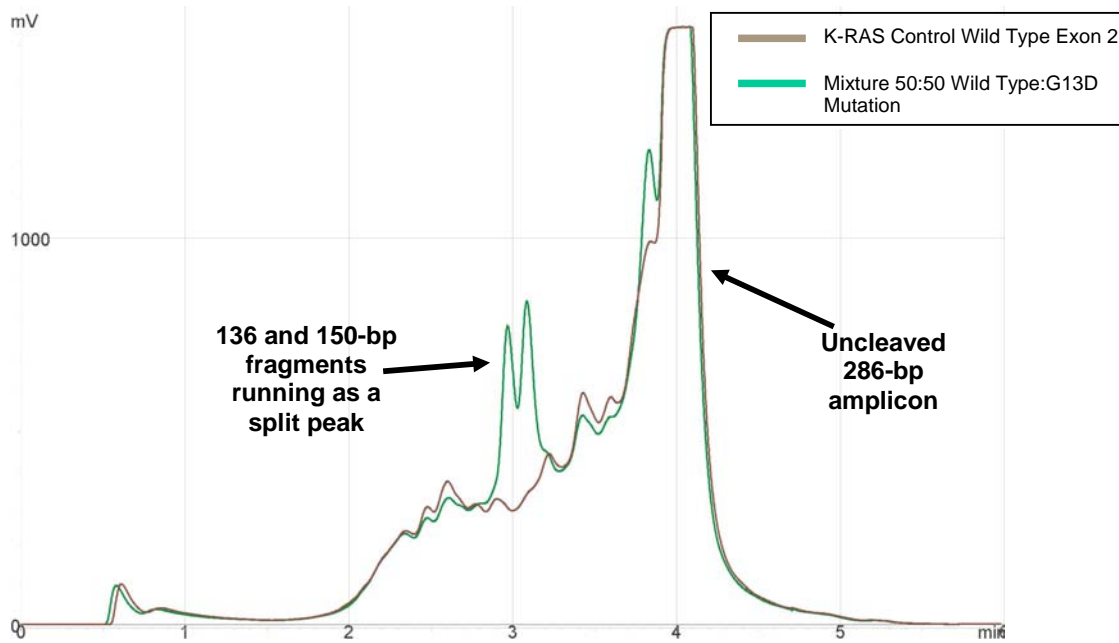


Figure 4 shows SURVEYOR Nuclease digestion products from the 286-bp exon 2 codon 13 mutation amplicon heteroduplexes elute as a double peak. The templates used in this PCR were the K-RAS Control: Wild-Type Exon 2 and K-RAS Positive Control Codon 13. This G13D mutation produces G-T and C-A heteroduplexes which yield 136 & 150-bp fragments after SURVEYOR Nuclease digestion; these fragments' peaks separate to produce a characteristic double peak. Digestion products were analyzed on a WAVE HS system.

This kit is designed to demonstrate clearly a distinctive WAVE trace for codon 12 and 13 mutations; this gives the user the option of internally validating the ability to deduce a sample's mutation status without the need for sequence confirmation.

Performance Characteristics

Level of Detection of SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD*

Validation of the SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* using plasmid clones of all common K-RAS exon 2 mutations has shown that SURVEYOR Nuclease peaks can be detected in a mixture of 1% mutant to 99% wild-type.

The results below show the WAVE HS System traces of dilution series for example mutations in each of codons 12 and 13 and the sequencing electropherograms of selected dilutions.

K-RAS Exon 2 G12S Mutation Level of Detection Dilution Series

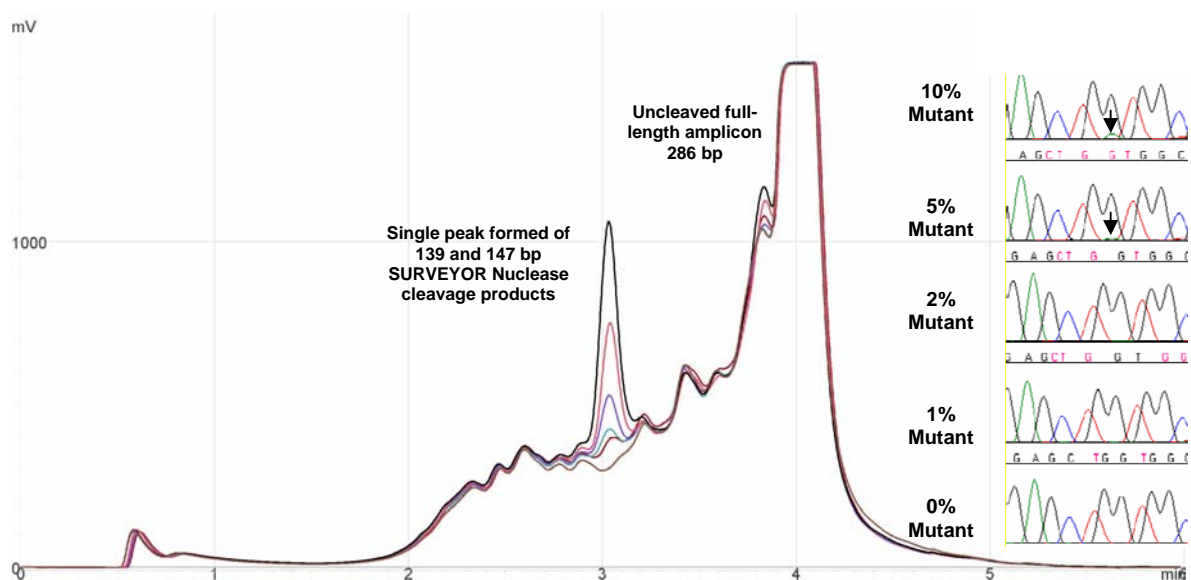
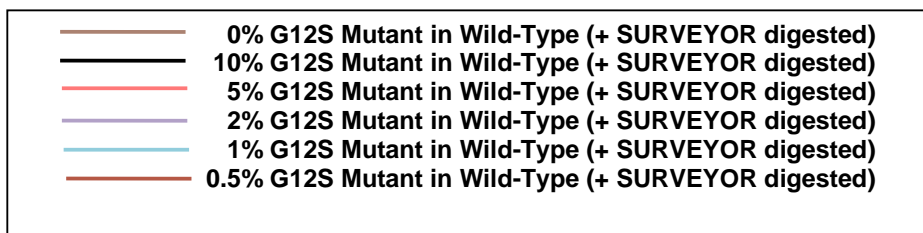


Figure 5 SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* Titration of the K-RAS Exon 2 G12S mutation

Varying mutant levels were prepared by the mixing of K-RAS Control: Wild-Type Exon 2 (PN 710141) and K-RAS Positive Control Codon 12 (PN 710143), then heating and cooling the mixture to form heteroduplexes. These mixtures were then cleaved with SURVEYOR Nuclease and analyzed on the WAVE 4500 HT HS System. **NOTE:** to achieve a 90% Wild Type, 10% Mutant, a blend of 80% PN 710141 and 20% PN 710143 (see page 11) is prepared. Because the G12S mutation is located near the centre of the Exon 2 amplicon, the two SURVEYOR Nuclease cleavage products have very similar retention times and form a single peak. Also note that the majority of the amplicon mix consists of wild-type homoduplexes that are not cleaved by SURVEYOR Nuclease. Limit of detection of the G12S mutant amplicon is 1% with SURVEYOR + WAVE HS.

Limit of Detection Sequencing Results for K-RAS Exon 2 G12S Mutation

Electropherograms show the sequencing results for PCR products analyzed by SURVEYOR Nuclease. Automated sequencing calling of both forward and reverse strands often fails to detect the G12S mutation in mixtures with wild-type below 10%. Together with the SURVEYOR Nuclease results, it is possible to more confidently interpret the 5% mutant sequencing electropherograms for codon 12.

K-RAS Exon 2 G13D Mutation Level of Detection Dilution Series

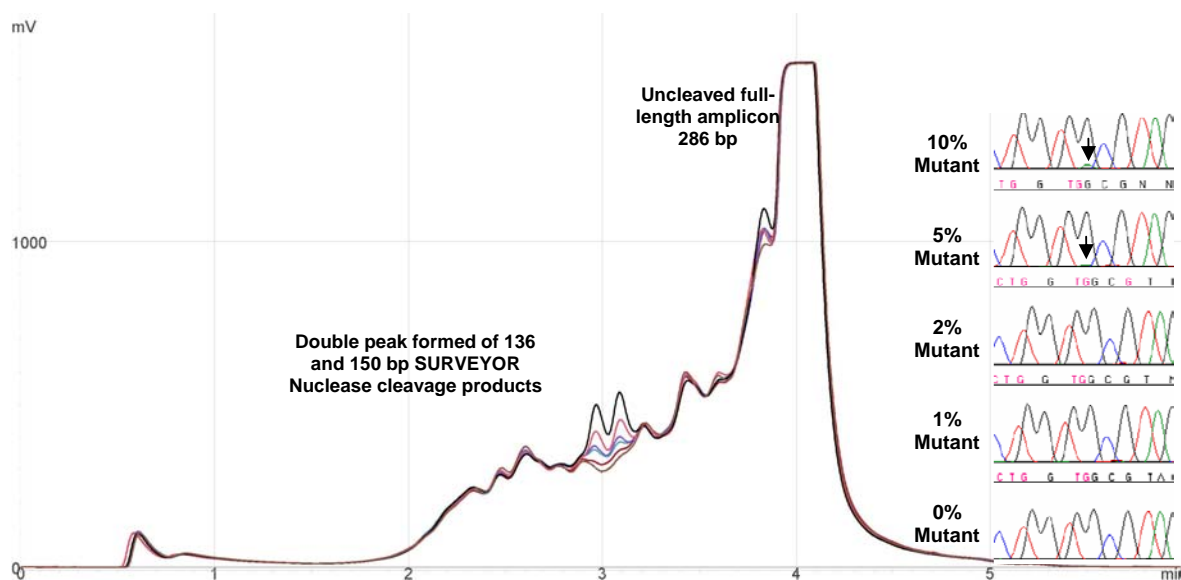
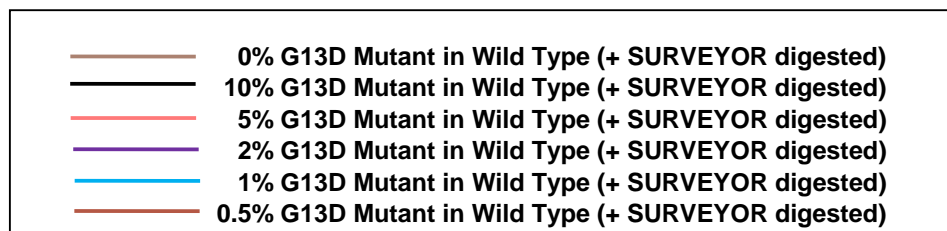


Figure 6 SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* Titration of the K-RAS Exon 2 G13D Mutation

Varying mutant levels were prepared by the mixing of K-RAS Control: Wild-Type Exon 2 (PN 710141) and K-RAS Positive Control Codon 13 (PN 710144), then heating and cooling the mixture to form heteroduplexes. These mixtures were then cleaved with SURVEYOR Nuclease, and analyzed on the WAVE 4500 HT HS System. **NOTE:** to achieve a 90% Wild Type, 10% Mutant, a blend of 80% PN 710141 and 20% PN 710144 (see page 11) is prepared. Because the G13D mutation is slightly further from the centre of the exon 2 amplicon than the G12S mutation (see Figure 5), the two SURVEYOR Nuclease cleavage products have different retention times and form a double peak. Note that most of the amplicon mix consists of wild-type homoduplexes that are not cleaved by SURVEYOR Nuclease. Limit of detection of the G13D mutant amplicon is 1% with SURVEYOR + WAVE HS.

Limit of Detection Sequencing Results for K-RAS Exon 2 G13D Mutation

For comparison, electropherograms showing the sequencing results for PCR products at the varying mutant levels are shown. Automated sequencing calling of both forward and reverse strands often fails to detect the G13D mutation in mixtures with wild-type below 10%. Together with the SURVEYOR Nuclease results it is possible to more confidently interpret the 90:10 wild-type:mutant (5% mutation) sequencing electropherograms for codon 13 as containing a mutation.

Interpretation of Low Percentage Mutation Samples

Although the internal and external validation studies of the SURVEYOR Scan K-RAS Kit have shown that detection of mutations at 1% of wild-type is realistic, this is dependent upon the quality of a given trace's baseline. Poor quality genomic DNA or PCR reactions, inadequate DNasep column washing procedures (see Washing Procedure page 30) or a sub-optimally WAVE System set-up can all result in a more irregular baseline and make discrimination of minor peaks over baseline more challenging.

The traces below (Figures 7 and 8) show the expanded regions of traces with low level mutations and negative control baselines.

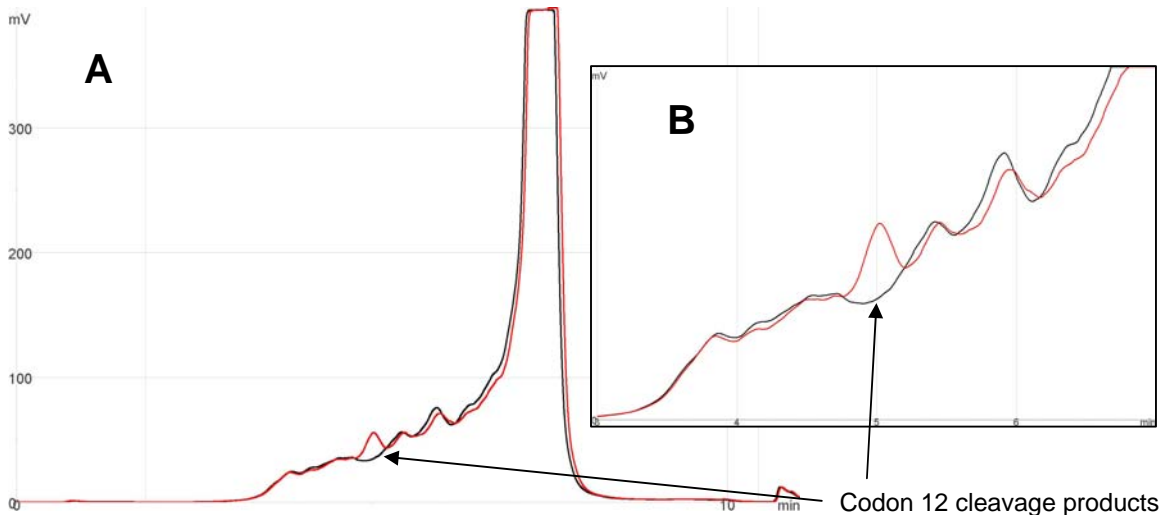


Figure 7 Low level detection of Codon 12 mutation. Red line = sample with low level codon 12 mutation. Black line is wild-type control. Figure 7B is a magnified region of the region that shows the SURVEYOR Nuclease cleavage products. Note the baseline noise with similar amplitude peaks to the cleavage fragment peaks is consistent between the test and control traces.

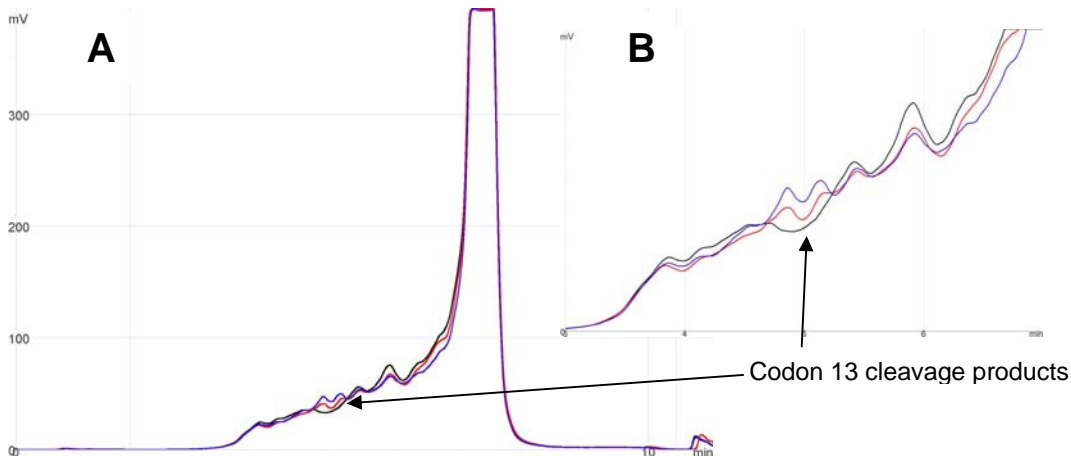


Figure 8 Low level detection of Codon 13 mutation. Red and blue lines = samples with low level codon 13 mutations. Black line is wild-type control. Figure 8B is a magnified region of the region that shows the SURVEYOR Nuclease cleavage products. Note the baseline noise with similar amplitude peaks to the cleavage fragment peaks is consistent between the test and control traces.

We would recommend that in the circumstances of there being any doubt about the presence or absence of SURVEYOR Nuclease digestion peaks the sample analysis should be repeated using the same sample genomic DNA template. If the presence of peaks is still suspected or indefinite the genomic DNA should be isolated afresh and re-analyzed. Recommendations to enhance signal to noise ratios include:

- Selecting FFPE slides in which there is a high concentration of tumor cells.

- Micro-dissection to increase the proportion of tumor to normal cells.
- Checking that genomic DNA meets the purity criteria outlined in **Template Considerations** on page 7.

Low percentage mutation loads will be equally difficult to confirm by DNA sequencing.

Limitations of the Assay Procedure

Contaminating substances carried over from extraction of formalin-fixed paraffin-embedded samples may interfere with the PCR amplification and SURVEYOR Nuclease digestions procedures. The quality control procedures outlined in Quality Control of PCR Products p10 will ensure that the extracted DNA is suitable for use in this kit.

This kit has been validated for use with formalin-fixed paraffin-embedded colorectal cancer tumor biopsy samples. It has not been validated for use either with other cancer-types or with fresh or frozen biopsy samples.

For troubleshooting non-standard results and details of factors that can affect this assay, see the Troubleshooting Guide in Appendix A below.

Care must be taken to avoid carryover and cross-contamination with this kit. The extreme sensitivity of the assay method requires precautions to be taken at the following points:

- Ensure that all samples are handled such that cross-contamination between samples cannot occur
- Ensure that the kit's plasmid controls are handled separately from test samples at all stages of the assay
- Ensure that sample pipetting into 96-well plates does not allow sample contamination of adjacent wells due to splashing during mixing or by not changing pipette tips.

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Appendix A

Troubleshooting Guide

Effective use of the SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* depends upon successful completion of a number of steps. One of the most critical is PCR amplification that must result in the production of specific, uniform-sized DNAs in sufficient quantity to be detected after hybridization and cleavage. This in turn is dependent on the quality of the initial sample. Performing the assay with DNA which does not meet the quality and quantity criteria is not recommended.

Note: If you are a first time user of SURVEYOR Nuclease, perform the experiments in the section **Using K-RAS Control Plasmid DNAs** (pages 11) after reading and understanding the section Step-by-Step Instructions (p7). Please have the results from the K-RAS Control Plasmid DNAs before contacting Transgenomic Technical Support.

This Troubleshooting Guide covers a list of issues that you might encounter when using the SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD* and suggestions on how to resolve them.

Problem 1 – Low PCR yield or no PCR product

POSSIBLE CAUSE	SOLUTION
Poor quality of DNA template	Repeat purification of DNA; review purification method used.
Thermocycler not calibrated	Check calibration of thermocycler
Not enough template	Increase the template amount.

Note: High quality DNA from FFPE should be used. The DNA should have a concentration of 25 ng/μL as determined by absorbance at 260 nm, have an absorbance ratio at 260/280 nm of >1.8 and be >90% DNA (i.e. free of most tRNA and rRNA contamination as judged by appearance on an agarose gel). Store DNA samples at -20 °C.

Analysis of DNA template extracted from paraffin-embedded tissue requires several precautions to be taken. The extracted DNA can be treated with uracil DNA glycosylase to prevent amplification of DNA fragments containing deaminated C residues. Often a high percentage of the A₂₆₀ adsorbing material extracted from paraffin-embedded tissue is not amplified well during PCR. Using a larger amount of starting DNA will often help to produce a suitable amplification product.

Problem 2 – Multiple PCR products

POSSIBLE CAUSE	SOLUTION
Annealing temperature too low	Check calibration of thermocycler.

Note: PCR should produce a sufficiently high yield (>20 ng/μL) of a **SINGLE** amplified species of the correct size. **The DNA Polymerase and the DNA Polymerase 10X PCR Buffer supplied with this kit must be used for PCR.** Amplicons from Controls should be digested with SURVEYOR Nuclease and run to exclude spurious background noise by visual comparison of chromatogram profiles (see **Examples of Results** page 13, Figures 3-4). Examine each amplified DNA product before digestion by gel electrophoresis or WAVE HPLC to be sure it is a single species of the expected size.

Problem 3 – No cleavage products observed upon analysis after SURVEYOR Nuclease treatment of heteroduplexes

POSSIBLE CAUSE	SOLUTION
Proportion of mismatch target too low	Check assay by using Controls.
Inefficient formation of heteroduplexes	Follow correct Hybridization Procedure. Use freshly hybridized DNA in SURVEYOR Nuclease digests.
Inactive SURVEYOR Nuclease	Perform the Control reaction to verify enzyme performance.

Note: SURVEYOR Nuclease predominately cleaves at mismatches in heteroduplexes. The proportion of heteroduplex to homoduplex in the hybridized sample will determine the SURVEYOR Nuclease digestion signal. If the K-RAS mutation is present at a very low concentration in the genomic DNA sample, the signal may be too low to give a positive result.

It is important to ensure that the hybridization step is included in the thermocycler program (see **Amplification Protocol** page 9) in order to maximize the efficiency of heteroduplex formation. Heteroduplexes are very inefficiently formed during standard PCR reactions.

Note: the signal to noise ratio is generally high enough to detect mutations present at a low percentage of the total DNA template; it is possible to detect 1% to 2% mutant DNA. Figures 5 and 6 (pages 15-16) show detection of K-RAS Exon 2 codon 12 and 13 mutations present (2-18% heteroduplex) with a WAVE HS system. Figures 3-4 (pages 13-14) show the digestion products generated with homoduplex and heteroduplex K-RAS Positive Control DNAs (included in this kit) on a WAVE HS System. The mutation-specific cleavage products are clearly seen as two new peaks eluting with the expected sizes that can be estimated relative to the DNA size marker.

Caution: commercially available PCR buffers vary dramatically in content and the formulations are often not defined by suppliers. Several buffers are **NOT** compatible with SURVEYOR Nuclease due to pH or the presence of additives, surfactants or other proprietary ingredients. **DO NOT use any other polymerase or 10X polymerase buffers than those provided in the kit.**

Problem 4 – High background after SURVEYOR Nuclease treatment

POSSIBLE CAUSE	SOLUTION
Suboptimal hybridization step	Do the following: 1. Make sure the DNA concentration is in the range of >25 ng/μL. 2. Repeat the hybridization step, taking care to follow the Hybridization procedure.
DNA amount too low	Check yield and quality of template DNA
Nonspecific PCR products	Check yield and quality of template DNA.
SURVEYOR Enhancer W2 and/or SURVEYOR Enhancer Cofactor have lost activity	Check Expiry Date of kit.

Note: SURVEYOR Nuclease occasionally nicks double-stranded DNA at random matched sites, which produces background after digestion¹⁸. This activity is suppressed by SURVEYOR Enhancer W2 and its cofactor without otherwise negatively affecting the mismatch cleavage reaction. SURVEYOR Enhancer W2 and cofactor are included in this kit and should always be used.

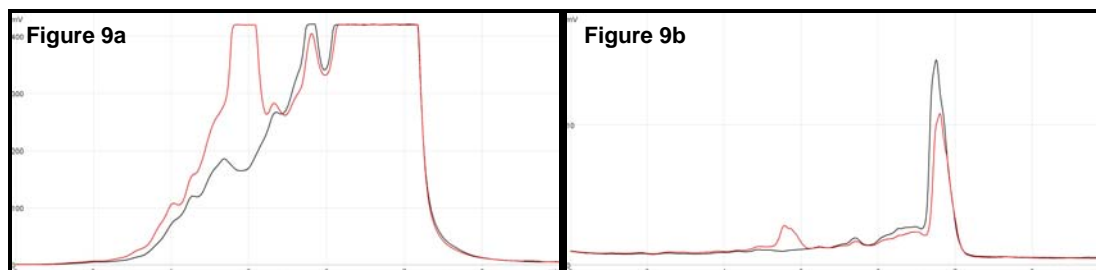
Where this nicking still occurs, generating minor peaks, comparing control digests of homoduplexes with sample digests will allow these to be recognized and normalized with Navigator Software.

Problem 5 – SURVEYOR Nuclease digestion peaks in negative controls

POSSIBLE CAUSE	SOLUTION
Contamination of kit contents with K-RAS amplicons or plasmid controls.	Dispose of all kit components and use fresh kit. Contact Transgenomic Technical Support to discuss potential causes and sources of contamination.

Problem 6 – Tops of SURVEYOR Nuclease digestion peaks truncated on WAVE HSD fluorescent trace

POSSIBLE CAUSE	SOLUTION
The high sensitivity detector has an internal feedback algorithm which monitors the amount of light going through the flow cell. The more light that enters the flow cell, the lower the detector cut off value (mV) but the higher the sensitivity.	Changing the emission wavelength would solve this issue but at the expense of losing sensitivity. We recommend referring to the UV detector chromatographic trace. If the fluorescent detector peaks are truncated in the chromatogram, then the UV trace will give you a clear readable signal. See examples in Figures 9a and 9b below. Alternatively, a smaller volume of sample can be re-injected and the fluorescent signal intensity should decrease.



Figures 9a and 9b. When finding truncated peaks using the HSD Fluorescent Detector, the UV Detector output can also be used for sample analysis

The traces above show the same genomic DNA samples' output from both the Fluorescent (9a) and UV (9b) Detectors. In each figure the red line is a G12V mutation sample; the black line is a wild-type reference sample. The high quantity of DNA loaded and analyzed using the fluorescent detector output in Figure 9a leads to truncated peaks for both the homoduplex and SURVEYOR Nuclease digestion peaks. The corresponding UV detectors trace shows the SURVEYOR Nuclease digestion peaks clearly as well as the uncut homoduplex peaks.

Appendix B

WAVE System INITIAL Setup/Cartridge Calibration (Navigator Software) for SURVEYOR Nuclease Applications

1. Use only WAVE Optimized buffers.
2. Purge Buffers A and B along with Solution D.
3. Prime the injection syringe (front panel of autosampler on 3500, through software on 4500).
4. Prime the HSD pump.
5. Under Setup on the drop down menu bar, select Module Setup.
6. Highlight the UV detector in the instrument box.
7. Verify that the detector wavelength is set to 260 nm.
8. Highlight the fluorescence detector in the instrument box.
9. Verify that the detector excitation wavelength is set to 492 nm and the emission wavelength is set to 526 nm (HS Staining Solution I).
10. Create new project (if desired) or open existing project using the Navigator Software drop down menu.
11. If a new project was created, a new sample tray needs to be created (ensure that the correct tray type is selected).
12. Create two blank injections using the universal linear application type.
 - a. On the speed plate, left click on vial 96 to highlight vial (assuming 96-well tray type is used).
 - b. Right click on vial to open injection page.
 - c. Change the application type to universal linear.
 - d. Change the cleaning option to fast clean (if applicable) or active clean.
 - e. Verify that the oven temperature is 50.0 °C in the method.
 - f. Change the volume to 0 µL.
 - g. Change the number of injections to 2.
 - h. Left click on the [Generate] button at the lower left.
13. Run injections.
14. Create three 5 µL injections of WAVE DNA Sizing Standard (Transgenomic part # 560078) using the universal linear application type.
 - a. Place sample of the WAVE DNA Sizing Standard in a 0.2 mL-PCR tube in position 96.
 - b. On the speed plate, left click on vial 96 to highlight vial (assuming 96-well tray type is used).
 - c. Right click on vial to open injection page.
 - d. Change the application type to universal linear.
 - e. Change the cleaning option to fast clean (if applicable) or active clean.
 - f. Verify that the oven temperature is 50.0 °C in the method.
 - g. Change the volume to 5 µL.
 - h. Change the number of injections to 3.
 - i. Left click on the [Generate] button at the lower left

15. Run injections.
16. Once the runs are complete, view chromatograms to ensure retention time stability of the 80- and 587-bp peaks.
 - a. The retention time of the 80-bp peak and the 587-bp peak of the WAVE DNA Sizing Standard should be within 0.1 min from run to run.
17. Perform cartridge calibration before running real samples.
 - a. Under the Analysis Tab, click on the [Select Results] button.
 - b. Find the third injection of the WAVE DNA Sizing Standard. Open the project and tray using the file navigation on the left, and then use the injection display on the right to find the injection.
 - c. Highlight the third WAVE DNA Sizing Standard injection and then click the [Add Selected Results] button.
 - d. Right click on the chromatogram.
 - e. Select [Chart].
 - f. Under the Peak Labels Option, select the Peak Labels Box and use the drop down menu to select [Peak Retention Time].
 - g. Click on [Apply].
 - h. Click on [OK].
 - i. Using the menu bar, use the Setup drop down menu and select [Cartridge Calibration].
 - j. Input the retention time values displayed on the chromatogram for the corresponding peak of the WAVE DNA Sizing Standard.
 - k. Select [Plot New Calibration].
 - l. Select [Accept New Calibration].

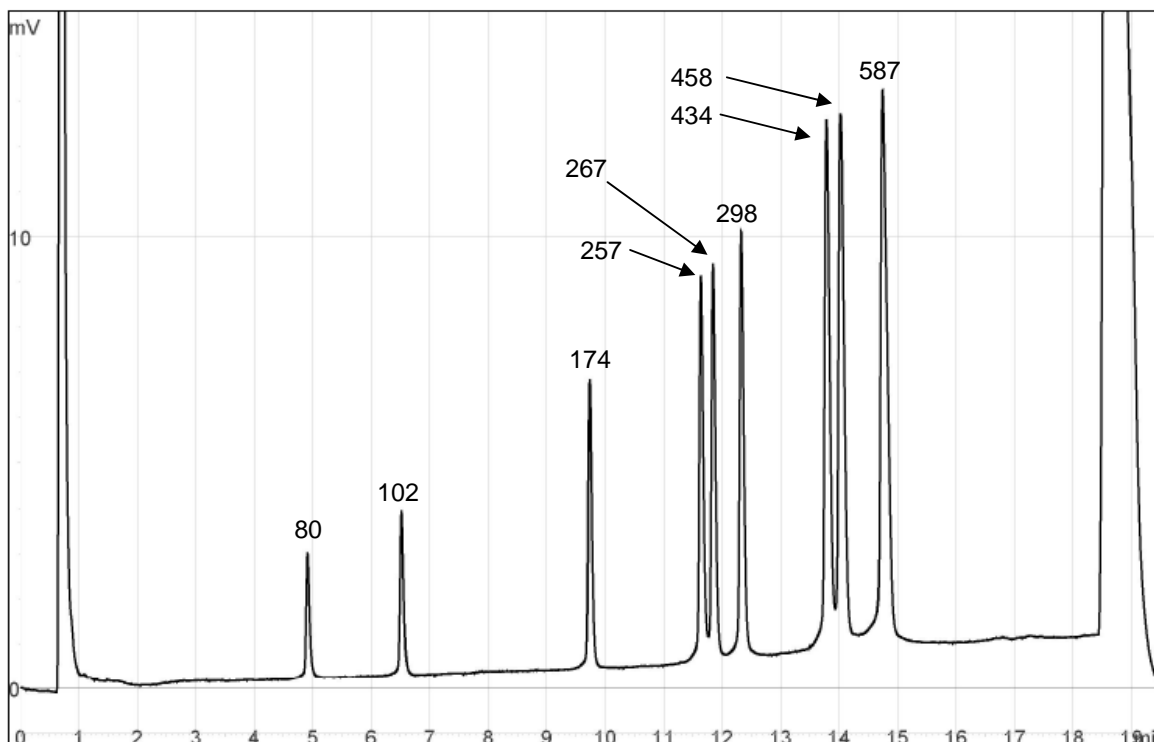


Figure 10. Typical WAVE DNA Sizing Standard analysis using the Universal Linear Method Type from Navigator Software (WAVE 4500 HT HS example).

WAVE HS System Parameters for K-RAS Protocol

We recommend downloading for installation into Navigator Software the K-RAS exon 2 CE Protocol Template. Refer to the K-RAS Installation Guide at:

<http://www.transgenomic.com/sp/sw/nav/K-RAS%20Protocol/K-RAS%20ProtocolCEIVD.asp>

For manual set-up of the WAVE HS System, or to check for correct installation of the K-RAS exon 2 CE Protocol Template, please refer to the following parameters for gradient prediction:

Flow rate: 1.2 mL/min
 Oven Temperature: 45.0 °C
 Application Type: dsDNA multiple
 Minutes/100 bp: 0.95
 Number of segments: 15
 Minimum base pair: 40 bp
 Maximum base pair: 400 bp
 Cartridge Clean: Fast Clean
 Cartridge: DNASep HT

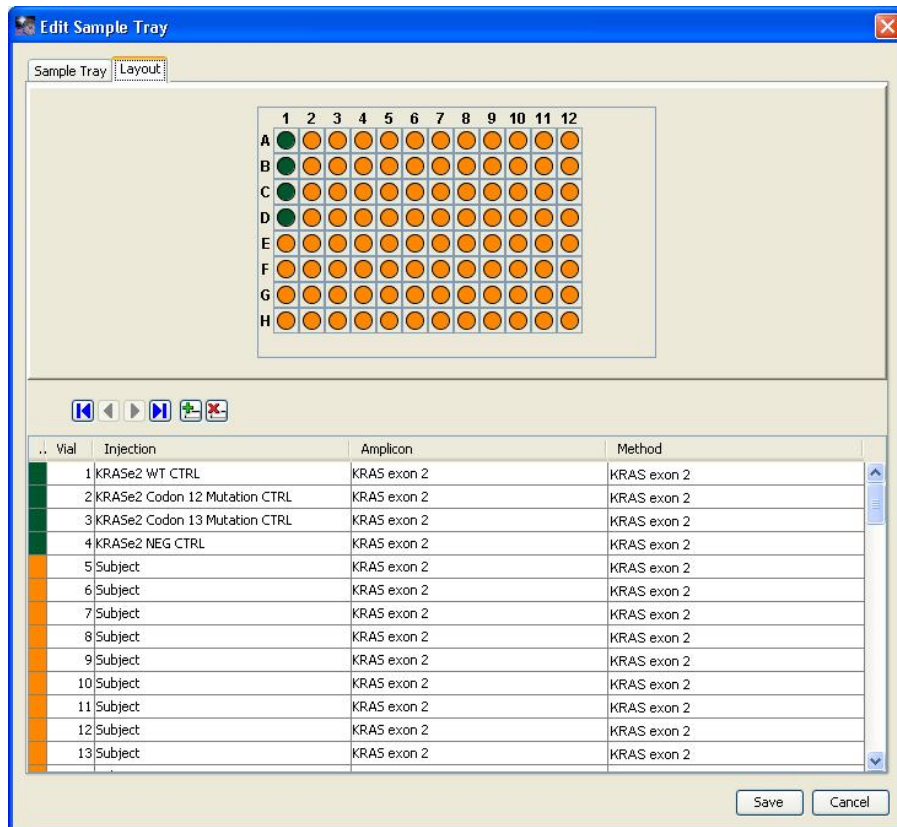


Figure 11 Plate set-up screen shot; 92 samples and controls

The K-RAS exon 2 CE Protocol Template populates the injection table as illustrated above. A total of 92 Exon 2 samples (orange wells) can be analyzed on a single plate. In addition, three controls for exon 2 (green wells) and a negative control are run for each plate.

Instructions For Use of the SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD*

Note that the injection table is set up in the column format.

- The K-RAS Control: Wild Type Exon 2 is placed in vial 1 (A1 on the injection table).
 - The K-RAS Positive Control Codon 12 is placed in vial 2 (B1 on the injection table).
 - The K-RAS Positive Control Codon 13 is placed in vial 3 (C1 on the injection table).
 - The negative control (no DNA added) is placed in vial 4 (D1 on the injection table).
1. To create a new protocol, go to [File] from the drop down menu bar, select [Protocol], then [Template].

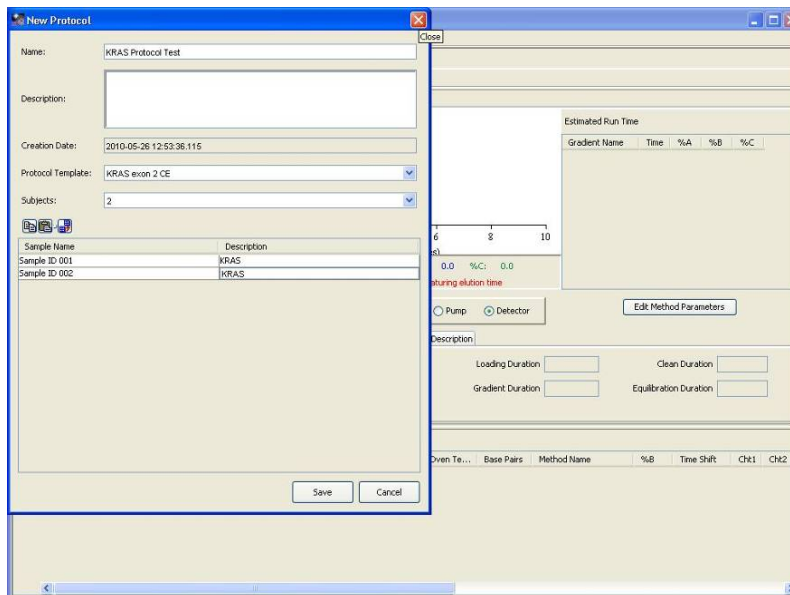


Figure 12 New Protocol Set-Up

- a) Name the protocol.
 - b) Ensure that the Protocol Template is "KRAS Exon 2 CE".
 - c) Enter the number of Subjects/Samples to be analyzed.
 - d) Input unique Sample Names for each of the Samples and select [Save].
2. To create the injection table, select a new Tray Type.
 - a) Input the Tray Name.
 - b) Select the Tray Type. The Tray Type must be in column format. If no tray is available, see the Navigator Manual for troubleshooting.
 - c) Select the K-RAS Kit Tray Template and select [OK]

Instructions For Use of the SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD*

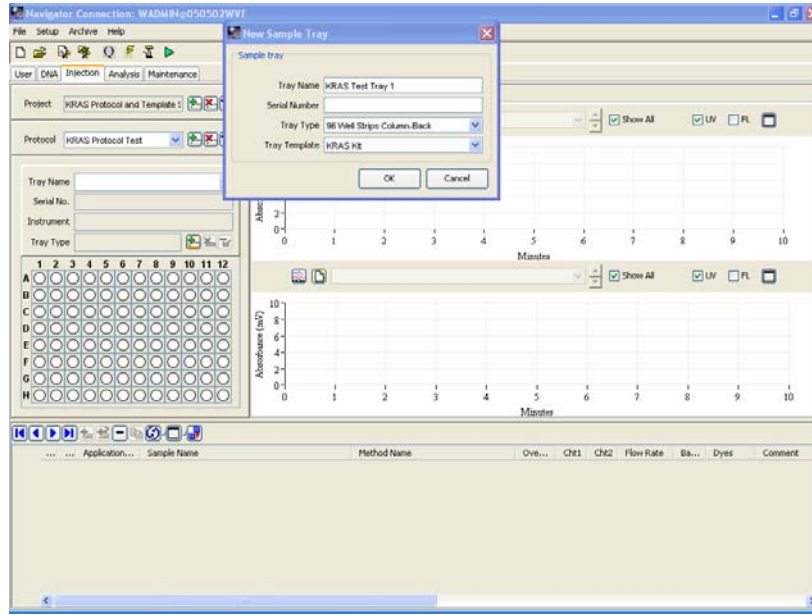


Figure 13 Setting up a New Sample Tray

The tray table will then be populated.

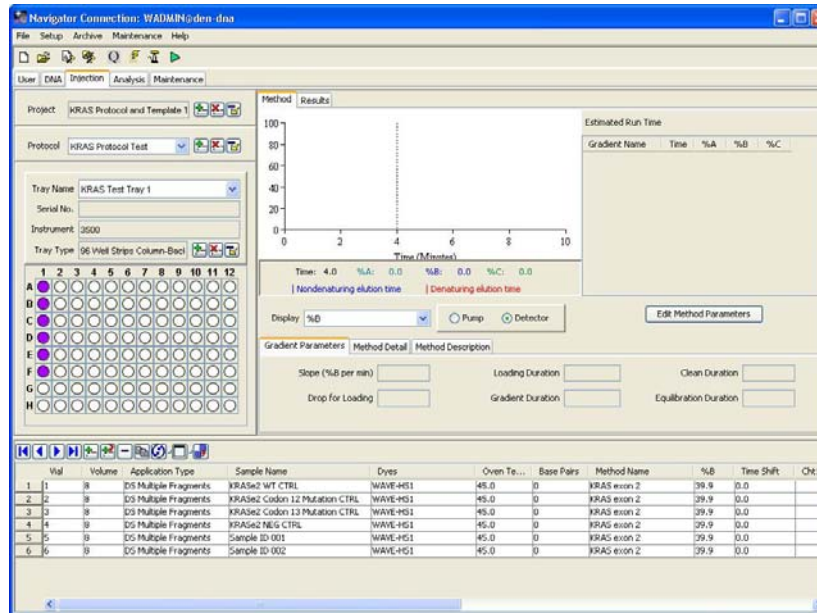


Figure 14 A Populated Sample Tray

- The above screen shot shows the population of the injection table using the K-RAS exon 2 CE template for the analysis of 2 samples.
- Sample 1 for K-RAS exon 2 should be placed in position E1 (vial 5).

Instructions For Use of the SURVEYOR Scan K-RAS Mutation Detection Kit *CE IVD*

5. In order to bring the HSD pump to operating pressure as well as equilibrate the system, a blank injection must be run prior to sample analysis. To do this:
 - a) Under [Setup] on the drop down menu, select [Project Defaults].
 - b) Check the Run box under Equilibrate Cartridge. One blank injection is sufficient.

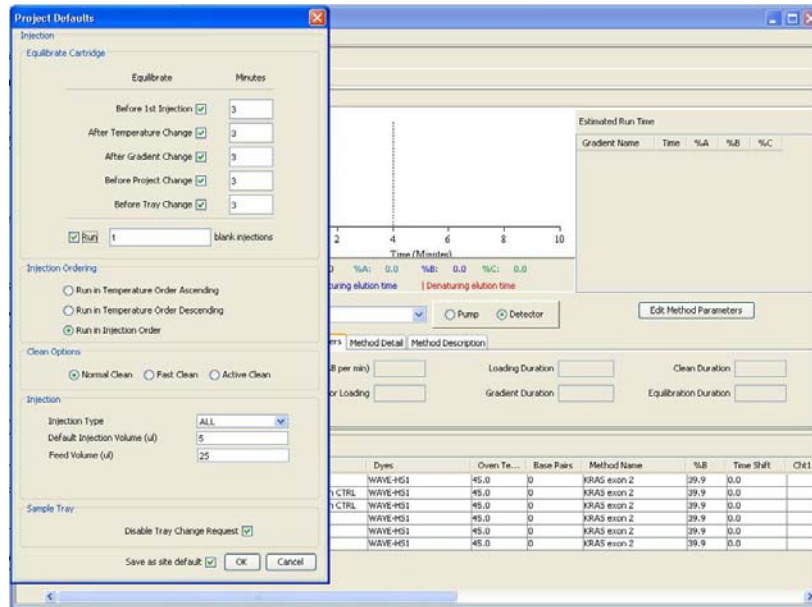


Figure 15 Running a Blank Injection

6. Vial 96 (position H12) can be used for the WAVE DNA Sizing Standard. Navigator Software Protocol function cannot be used to generate injections for the sizing standard on each of the gradients used for K-RAS analysis. To create injections of the sizing standard, duplicate two rows from the injection table that uses the K-RAS exon 2 CE method. Change the Sample Name to Sizing Standard.
7. Highlight the two sample rows with the sizing standard and run the two selected injections. Press the run button again to run the rest of the samples (all injections in tray). A blank injection will be run prior to the sizing standards.

Gradient for K-RAS Exon 2

(maximum fragment length = 400 bp)

Step	Time	%A	%B
Loading	0.0	65.0	35.0
40 bp	0.5	60.1	39.9
66 bp	0.7	54.8	45.2
92 bp	1.0	51.1	48.9
118 bp	1.2	48.3	51.7
144 bp	1.5	46.2	53.8
170 bp	1.7	44.5	55.5
196 bp	2.0	43.1	56.9
222 bp	2.2	41.9	58.1
248 bp	2.5	40.9	59.1
274 bp	2.7	40.1	59.9
300 bp	3.0	39.4	60.6
326 bp	3.2	38.7	61.3
352 bp	3.5	38.2	61.8
378 bp	3.7	37.7	62.3
404 bp	4.0	37.3	62.7
Start Clean	4.1	65.0	35.0
Stop Clean	4.2	65.0	35.0
Start Equilibrate	4.3	65.0	35.0
Stop Equilibrate	4.4	65.0	35.0

Maintenance of DNASep HT Cartridges

Washing Procedure

When injecting SURVEYOR Nuclease digests onto DNASep HT Cartridges in a WAVE System, the recommended cleaning options are either ACTIVE CLEAN or FAST CLEAN. **A normal clean is not sufficient.**

Note the following:

- Every 100 injections of SURVEYOR Nuclease digests should be followed by a HOT WASH. To perform a HOT WASH, at 80 °C pump 100% Solution D (75% (v/v) ACN) for 15 minutes, followed by a wash of a 1:1 mix of WAVE Optimized Buffers A and B for 30 minutes through the system. This HOT WASH should also be performed at the end of each batch of SURVEYOR Nuclease digest runs irrespective of whether 100 injections have been reached or not, e.g. when temporary cessation of cartridge use or a change to DHPLC analysis is anticipated.
- The in-line filter must be changed after every 500 injections of SURVEYOR Nuclease digests. The in-line filter should also be changed if the pressure becomes too high (>1500 PSI for DNASep HT Cartridges).
- After every 500 injections a REVERSE HOT WASH should be performed. Refer to **Performing a REVERSE HOT WASH on a DNASep HT Cartridge** section below.

WARNING! Failure to follow these procedures will lead to high column pressure and deteriorating column performance.

Performing a REVERSE HOT WASH on a DNASep HT Cartridge

To perform a REVERSE HOT WASH:

1. Remove the DNASep HT Cartridge and reinsert it in the reverse orientation in the flow path.
2. Remove the in-line filter and substitute it with a union during the REVERSE HOT WASH.
3. Pump 100% Solution D (75% (v/v) ACN) for 30 minutes at 80 °C through the system at a flow rate of 0.9 mL/min.
4. Pump a wash of a 1:1 mix of WAVE Optimized Buffers A and B for 1 hour at 80 °C through the system at a flow rate of 0.9 mL/min.
5. Remove the union and insert a new in-line filter.

This REVERSE HOT WASH should be performed without the in-line filter. Insert a new in-line filter after the REVERSE HOT WASH and run the cartridge in the reverse direction for the next 500 injections.

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Transgenomic Inc operates a Quality Management System which complies with the requirements of ISO 9001:2008 for instrument and bioconsumable product manufacturing provided for genetic variation, mutation detection and other unique biomarker assays, as certified by BSI, certificate # FM 538914.

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