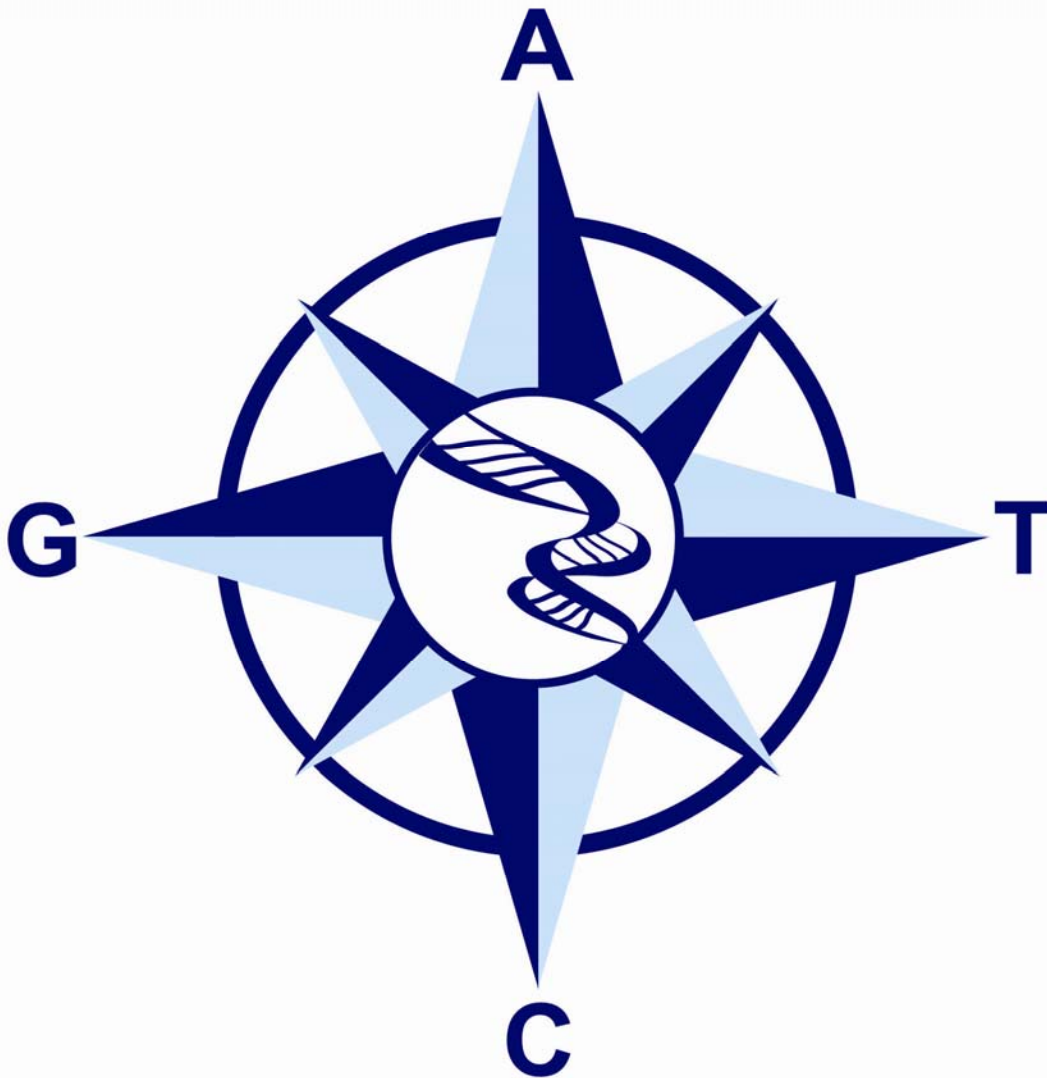




# Instructions for Use of the SURVEYOR® Scan K-RAS Mutation Detection Kit Exon 2

For use with Agarose Gel Electrophoresis



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## SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2*

### Introduction

This kit is designed to be used with Bio-Rad and Lonza agarose gel electrophoresis platforms and is supplied as a single box containing the components indicated below. This Instruction for Use is available as a download.

Transgenomic's SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* is a **For Research Use Only** 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 research laboratory by suitably trained personnel testing DNA extracted from formalin-fixed paraffin embedded tissues.

The SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* **should not** be used for diagnosis of colorectal or any other cancer or for decisions about therapeutic treatment.

The SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* 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.

## 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 (Erbix<sup>®</sup>) and panitumumab (Vectibix<sup>®</sup>), 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. This kit is designed for use in ongoing research on K-RAS exon 2 mutations and their effects drug efficacy.

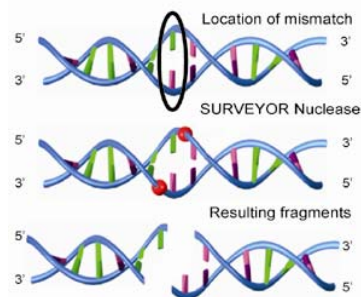
The SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* is a **For Research Use Only** kit for detecting all sequence and small insertion/deletion mutations in exon 2 of the K-RAS gene. Positive controls are supplied for exon 2 mutations in codons 12 and 13 that have been associated with the lack of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

This kit uses Transgenomic's proprietary SURVEYOR Nuclease technology and agarose gel electrophoresis to give simple and sensitive mutation detection, capable of detecting a mixture of 5% mutant in a background of 95% 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 *Exon 2* 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.

## Instructions For Use of the SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* Gel Electrophoresis

### 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 site<sup>1</sup>. Insertion/deletion mismatches and all base-substitution mismatches are recognized, but the efficiency of cleavage varies with the sequence of the mismatch<sup>1,2</sup>.



**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 genes<sup>5</sup>. Notably SURVEYOR Nuclease has been used to verify the presence of known mutations in a number of genes associated with renal cancer<sup>3</sup>, lung cancer<sup>4, 9, 10, 12-15</sup>, head & neck cancer<sup>6</sup>, leukemia<sup>7, 16, 17</sup>, endometrial cancer<sup>8</sup> and in radiotherapy outcome prediction<sup>11</sup>.

The SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* for Agarose Gel Electrophoresis has been designed to cleave mismatches in K-RAS exon 2 for subsequent analysis by agarose gel electrophoresis.

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

**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*.**

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](mailto: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>GAT 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*** for more details on DNA sequences of controls.

Instructions For Use of the SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* Gel Electrophoresis

## Components

The SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* 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 (710102) Volume Provided
703310	DNA Polymerase (2.5 U/ $\mu$ L)	100 $\mu$ L
703315	DNA Polymerase 10X PCR Buffer	1000 $\mu$ L
703065	dNTPs (10 mM)	500 $\mu$ L
710151F	K-RAS Primer: Exon 2 Forward (10 $\mu$ M) (2 tubes)	2 x 250 $\mu$ L
710151R	K-RAS Primer: Exon 2 Reverse (10 $\mu$ M) (2 tubes)	2 x 250 $\mu$ L
710160	SURVEYOR Nuclease W (2 tubes)	2 x 105 $\mu$ L
710161	SURVEYOR Enhancer W2	105 $\mu$ L
708049	SURVEYOR Enhancer Cofactor	105 $\mu$ L
708027	0.15 M MgCl <sub>2</sub> Solution	105 $\mu$ L
708030	Stop Solution	250 $\mu$ L
710141	K-RAS Control: Wild-Type Exon 2	40 $\mu$ L
710143	K-RAS Positive Control Codon 12	40 $\mu$ L
710144	K-RAS Positive Control Codon 13	40 $\mu$ L
482276	User Guide	Download from web site* <a href="http://www.transgenomic.com/pd/surveyor/SurveyorKRAS_RUO.asp">http://www.transgenomic.com/pd/surveyor/SurveyorKRAS_RUO.asp</a>

### Number of Samples that can be Tested with One Kit

The SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* 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. **Note:** batches of samples analyzed on Bio-Rad 2x 32-well (PN 161-3040) or Lonza 2x 16-well (PN 57032) agarose gels will require the four controls as well as a DNA sizing ladder run on each gel. Note that the recommended Lonza DNA Cassettes have two tiers of 16 sample wells plus one DNA Ladder well.

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 *Exon 2* includes the following:

Either

Bio-Rad ReadySub System (includes ReadySub-Cell GT Wide-Mini PN 170-4489; ReadyAgarose Wide Mini Gel PN 161-3040 containing a 3% (w/v) agarose gel plus ethidium bromide).

Or

Lonza FlashGel System (includes FlashGel Dock PN 57025; FlashGel DNA Cassette PN 57032 containing a 2.2% (w/v) agarose gel; FlashGel Loading Dye 5X Concentrate PN 50463; FlashGel DNA Marker 100-4000bp PN 50472)

1X TBE Buffer, e.g. Bio-Rad 10X Tris/Boric Acid/EDTA, 1 litre, PN 161-0733

## Instructions For Use of the SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* Gel Electrophoresis

5X DNA Loading Dye Concentrate, e.g. Bio-Rad Nucleic Acid Sample Loading Buffer PN161-0767  
UV Transilluminator for visualizing Ethidium bromide gels at 254 nm – only required for Bio-Rad gels  
Agarose gel photographic equipment capable of at least 3 megapixel image capture  
0.2 mL-PCR tubes  
2.0 mL-microcentrifuge tubes  
Micropipettors  
Pipette tips  
100-bp DNA mass ladder, e.g. Bio-Rad EZ Load 100 bp Molecular Ruler PV 170-8352  
Molecular biology grade water  
Ice bath  
Vortexer  
Microcentrifuge  
Thermocycler

### 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.

### 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** 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-710102 can be downloaded from

<http://www.transgenomic.com/lib/msds/710102.pdf>

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

The Bio-Rad agarose gel system uses ethidium bromide for visualization of DNA bands when using a UV transilluminator. Ethidium bromide is considered to be hazardous; for details on handling and disposal please consult the relevant Bio-Rad MSDSs.

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 *Exon 2* has been validated for use with DNA extracted from formalin-fixed paraffin embedded colorectal cancer tumor samples. To meet key

## Instructions For Use of the SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* Gel Electrophoresis

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 nm absorbance ratio should be >1.80.
- The template concentration for each sample should be 25 ng/μL.

Extracted DNA samples not intended for immediate analysis with this kit should be stored at -20 °C.

## Assay Procedure

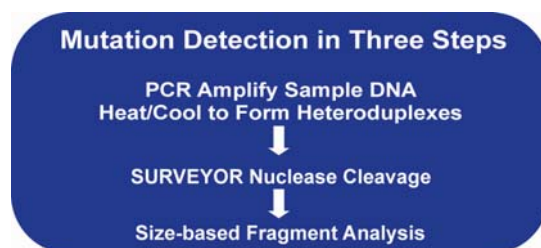
### Somatic Mutation Detection with the SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* - An Overview

Mutation detection and confirmation with SURVEYOR Nuclease involves three steps:

**Step 1** - Prepare PCR amplicons from mutant (test) and normal (reference) DNA, continuing on from the final PCR amplification cycle to melt all double strands and then cool slowly for optimal formation of hetero- and homoduplexes.

**Step 2** - Treat the annealed heteroduplex/homoduplex mixture with SURVEYOR Nuclease. The reference DNA alone, treated similarly, serves as a background control.

**Step 3** - Analyze the DNA fragments by agarose gel electrophoresis. The formation of new cleavage products, due to the presence of one or more mismatches, is indicated by the presence of additional bands. The migration distances of the cleavage products relative to the 100-bp DNA ladder indicate the size of the fragments and therefore the location of the mismatch or mismatches.



## Step-by-Step Instructions

### Lonza & Bio-Rad Agarose Gel Set-Up

Please see Lonza and Bio-Rad's User Manual for set-up procedures. **Maintain systems according to the manufacturer's instructions. Use only the manufacturers' gels designed for their respective systems. Run gels according to manufacturers' directions unless indicated otherwise.**

### 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.

Instructions For Use of the SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* Gel Electrophoresis

**Primer Considerations**

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

Amplicon		Sequence
Exon 2	Forward	<b>cggGTTTGTATTTAAAAGGTACTGGTGGAGT</b>
	Reverse	<b>cgggTTTATCTGTATCAAAGAATGGTCCT</b>

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

2. The amplicon sequences are as follows:
  - a. 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

**cgggtttgtattataaaaggtactggaggag**atttgatagtgattaaaccttatgtgtgacatgttctaataatagtcacattttcattatttt  
 attataag**GCCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTGGCGTA**  
**GGCAAGAGTGCCTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATC**  
**CAACAATAGAG**gtaaatctgttttaatatgcatattactggtgca**aggaccattctttgatacagataaa**cccg

**Amplification Protocol**

1. 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).
2. The forward and reverse primers (PN 710151F and 710151R, respectively) for each amplicon are supplied at 10 µM.
3. Remove 10 µM primers, 10 mM premixed dNTP solution and DNA Polymerase 10X PCR Buffer (PN 703315) from the freezer and thaw on ice.
4. Prepare Master Mix on ice.
5. Use the following table as a guide for preparing the Master Mix for K-RAS exon 2:

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

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

## Instructions For Use of the SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* Gel Electrophoresis

6. 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.
7. Label 0.2 mL-PCR tubes or wells of a 96-well plate with appropriate sample information.
8. Label a 2.0 mL-centrifuge tubes for Master Mix preparation.
9. Add required volume of molecular biology grade water to the 2.0 mL-centrifuge tube labeled Master Mix.
10. Vortex DNA Polymerase 10X PCR Buffer for ~10 s.
11. Add required amount of DNA Polymerase 10X PCR Buffer to the 2.0 mL-centrifuge tube.
12. Vortex 10.0 mM premixed dNTP working solution for ~10 s.
13. Add required volume of the 10.0 mM premixed dNTPs working solution to the 2.0 mL-centrifuge tube.
14. Add required volume of the K-RAS Primer: Exon 2 Forward to the 2.0 mL-centrifuge tube.
15. Add required volume of the K-RAS Primer: Exon 2 Reverse to the 2.0 mL-centrifuge tube.
16. Take DNA Polymerase (PN 703310) out of the freezer.
17. Centrifuge DNA Polymerase for ~10 s.
18. Vortex the DNA Polymerase for ~10 s.
19. Add required volume of DNA Polymerase to the 2.0 mL-centrifuge tube.
20. Cap the 2.0 mL-centrifuge tube containing Master Mix.
21. Vortex the 2.0 mL-centrifuge tube for ~30 s before use.
22. Store on ice until use.
23. Pipet 48.0  $\mu$ L 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.
24. To the appropriate wells add 2.0  $\mu$ L of each sample template DNA, each control template DNA (PN 710140, 710143, 710144) or template free control (water).
25. 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.
26. Vortex (~1/2 speed) for 30 s.
27. 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.

### Thermocycler Program for Amplification Protocol

1. 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

## Instructions For Use of the SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* Gel Electrophoresis

### Quality Control of PCR Products

1. Amplicon quality and quantity should be checked by gel electrophoresis 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 – Troubleshooting Guide**).
6. If no product is observed, ensure quality of input template DNA was sufficient (see **Appendix – 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 **Appendix - Troubleshooting Guide**.

### 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 MgCl<sub>2</sub> 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 MgCl<sub>2</sub> 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 MgCl<sub>2</sub> 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).

### For Bio-Rad's ReadySub Cell

13. Mix 5 μL of SURVEYOR Nuclease reaction with 1.25 μL 5X DNA Loading Dye Concentrate.

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14. Examine the gel visually for deformation or desiccation before loading into the ReadySub cell.
15. Load 6  $\mu\text{L}$  of each SURVEYOR Nuclease reaction/Dye mixture into separate wells of a ReadyAgarose Wide-Mini Gel. Load 0.25  $\mu\text{g}$  100-bp DNA Ladder as a DNA size standard.
16. Set voltage to 75V and run the gel for 45 minutes. By the end of the run the bromophenol blue dye front should typically migrate to the 2 cm mark on the gel tray.
17. Remove gel from ReadySub Cell, place on a UV transilluminator fitted with a 254 nm filter and photograph the gel. Note that Bio-Rad ReadyAgarose gels include Ethidium Bromide for DNA band visualization. Please refer to ***Analysis of K-RAS Exon 2 using SURVEYOR Nuclease.***

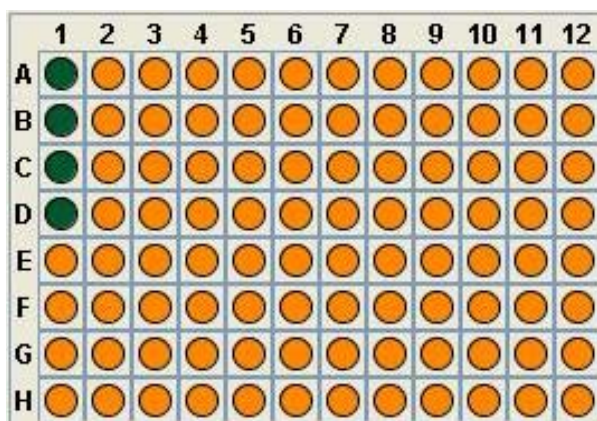
### For Lonza's FlashGel System

13. Mix 4  $\mu\text{L}$  of SURVEYOR Nuclease reaction with 1  $\mu\text{L}$  Lonza 5X DNA Loading Dye Concentrate (included in gel packages).
14. Load 5  $\mu\text{L}$  of the SURVEYOR Nuclease reaction/Dye mixture into separate wells of the DNA cassette pre-filled with distilled water. Load 5  $\mu\text{L}$  (~65 ng) of FlashGel DNA Marker as a DNA size standard.
15. Set voltage to 145V and run the gel for 10 minutes. NOTE that this is a lower voltage than generally suggested by the the manufacturer.
16. Remove the gel from the cassette, place on a dark reader (blue excitation/green emission and photograph the gel. Note that Lonza FlashGels include SYBRGreen for DNA band visualization. Please refer to ***Analysis of K-RAS Exon 2 using SURVEYOR Nuclease.***

**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 2 shows a 96-well plate layout with controls and 92 samples. Smaller batches of samples can be run, but the controls and 100-bp DNA ladder should still be run every time. There are sufficient control materials in the kit for all combinations of sample batch sizes to be used.



**Figure 2 Example of 96-well plate layout.** A total of 92 Exon 2 samples (orange wells) can be analyzed on a single plate. In addition, three controls for Exon 2 and a negative, no template control (green wells) are run for each plate.

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  $-20\text{ }^{\circ}\text{C}$  until the next step is carried out. However, exposure of any frozen sample to repeated freeze-thaw cycles should be avoided and storage at  $-20\text{ }^{\circ}\text{C}$  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 *Exon 2*

Control Plasmid DNAs are included in the kit to provide quality control checks at specific steps in the assay procedure. For the **Amplification Protocol**, 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 agarose gel images of these SURVEYOR Nuclease digested control amplicons provide guidance of where the codon 12 and 13 mutations, even at low levels, will migrate (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** or **Examples of Results**, consult the **Appendix - Troubleshooting Guide** or contact Transgenomic Technical Support before proceeding with further steps in the analysis of 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/ $\mu$ L.

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 sequence differences in the Positive Controls are highlighted in **Purple**. Capitalized letters that are not highlighted are non-coding cDNA regions.

## Instructions For Use of the SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* Gel Electrophoresis

### K-RAS Control: Wild-Type Exon 2

MD Loci: 10428 - 10706

Size: 286 bp

**cgggtttgtattaaaaggctactggaggag**atttgatagtgattaaccttatgtgtgacatgttctaataatagtcacatttcattat  
attataagGCCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTGGCGTA  
GGCAAGAGTGCCTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATC  
CAACAATAGAGgtaaactctgtttaaatatgcatattactggtgc**aggaccattcttfgatacagataaa**cccg

### K-RAS Positive Control Codon 12

MD Loci: 10428 - 10706

Size: 286 bp

**cgggtttgtattaaaaggctactggaggag**atttgatagtgattaaccttatgtgtgacatgttctaataatagtcacatttcattat  
attataagGCCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGAGCT[G/A]GTGGCG  
TAGGCAAGAGTGCCTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGAT  
CCAACAATAGAGgtaaactctgtttaaatatgcatattactggtgc**aggaccattcttfgatacagataaa**cccg

### K-RAS Positive Control Codon 13

MD Loci: 10428 - 10706

Size: 286 bp

**cgggtttgtattaaaaggctactggaggag**atttgatagtgattaaccttatgtgtgacatgttctaataatagtcacatttcattat  
attataagGCCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTG[G/A]CG  
TAGGCAAGAGTGCCTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGAT  
CCAACAATAGAGgtaaactctgtttaaatatgcatattactggtgc**aggaccattcttfgatacagataaa**cccg

Please follow instructions found in *Amplification Protocol*, *SURVEYOR Nuclease Digestion* and *Analysis of K-RAS Exon 2 using SURVEYOR Nuclease* for use of these controls.

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

## Gel Image Quality

The quality of the gel images is critical to the correct detection of bands of SURVEYOR Nuclease cleavage products. To assure that the gel image detection and photographs are of sufficient quantity use the kit's positive controls to prepare a 5% mutation to run using the kit.

This can be prepared by mixing PN 710141, K-RAS Control: Wild-Type Exon 2 with either PN 710143, K-RAS Positive Control Codon 12, or PN 710144, K-RAS Positive Control Codon 13, in a ration of 9:1 and using in SURVEYOR Nuclease digestion assays as outlined in this manual's section *SURVEYOR Nuclease Digestion*. Note that PNs 710143 and 710144 are mixtures of 50:50 wild-type and mutant DNA.

Compare the quality of the photographs prepared by your image system to those in *Figures 5 or 6* to assure that you can readily identify mutations as low as 5%.

## Interpretation of Results

### Analysis of K-RAS Exon 2 using SURVEYOR Nuclease

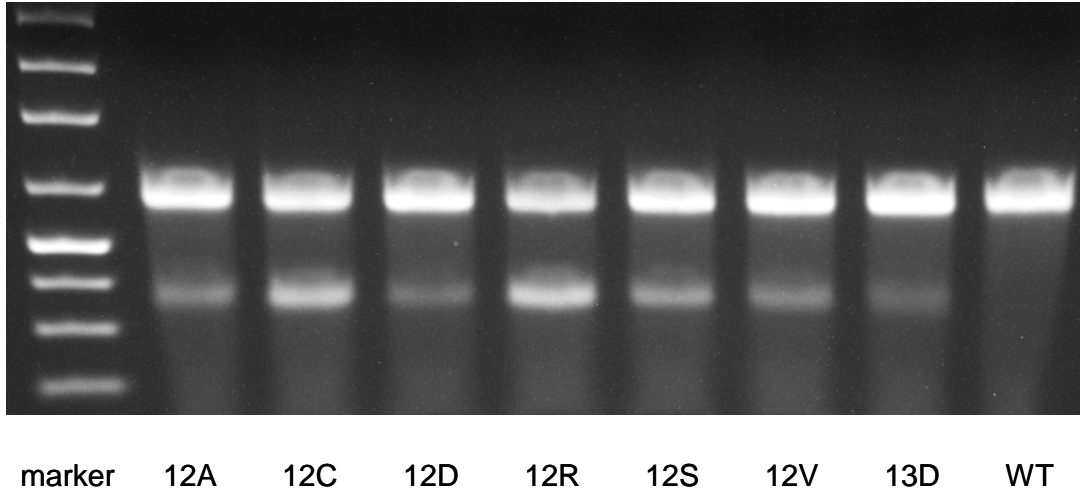
1. For set-up of the Lonza or Bio-Rad agarose gel electrophoresis systems please refer to the manufacturer's guidelines.

## Instructions For Use of the SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* Gel Electrophoresis

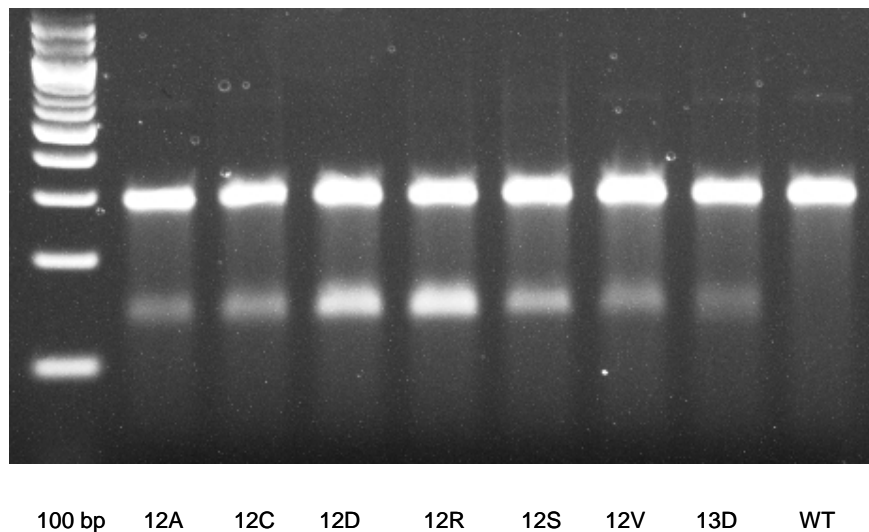
2. Please note that, for comparison/control purposes, always perform SURVEYOR Nuclease digestion on both the controls (wild-type and positive controls) and the sample DNAs and run on the same agarose gel.

### Examples of Results

Examples of results obtained using the SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* for Agarose Gel Electrophoresis 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 codons 12 and 13 amplicon heteroduplexes analyzed on a Lonza FlashGel System.** The templates used in this PCR were the K-RAS Control: Wild-Type Exon 2 and clones of K-RAS Exon 2 mutations G12A, G12C, G12D, G12R, G12S, G12V and G13D. The kit includes G12S and G13D mutation controls. These SURVEYOR Nuclease digestion fragments' bands are not resolved on this gel and run as a single band. The marker is a 100-bp DNA ladder; the most intense marker band is 400 bp.



**Figure 4 shows SURVEYOR Nuclease digestion products from the 286-bp exon 2 codons 12 and 13 amplicon heteroduplexes analyzed on a Bio-Rad ReadySub System.** The templates used in this PCR were the K-RAS Control: Wild-Type Exon 2 and clones of K-RAS Exon 2 mutations G12A, G12C, G12D, G12R, G12S, G12V and G13D. The kit includes G12S and G13D mutation controls. These SURVEYOR Nuclease digestion fragments' bands are not resolved on this gel and run as a single band. The marker is a 100-bp DNA ladder; the most intense marker band is 400 bp.

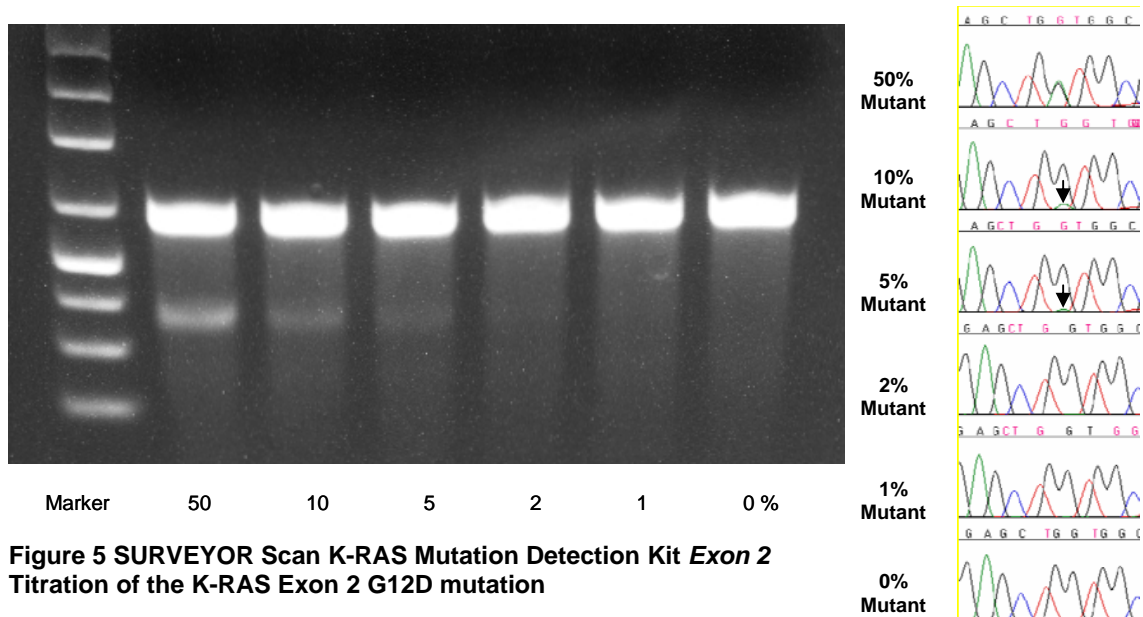
## Performance Characteristics

### Level of Detection of SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2*

Validation of the SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* using plasmid clones of all common K-RAS exon 2 mutations has shown that SURVEYOR Nuclease peaks can be detected in a mixture of 5% mutant to 95% wild-type.

The results below show the DNA gel electrophoresis results of dilution series for example mutations in each of codons 12 and 13 and the sequencing electropherograms of selected dilutions.

### K-RAS Exon 2 G12D Mutation Level of Detection Dilution Series



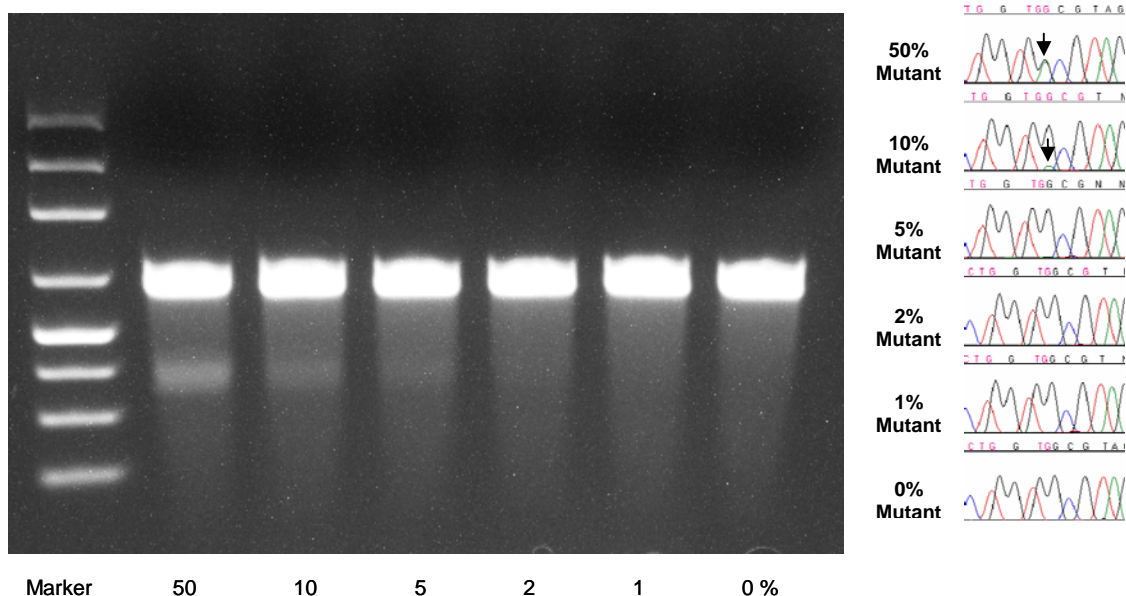
**Figure 5 SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* Titration of the K-RAS Exon 2 G12D mutation**

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 Lonza FlashGel System. **NOTE:** to achieve a 90% Wild-Type, 10% Mutant, a blend of 80% PN 710141 and 20% PN 710143 is prepared. Because the G12D mutation is located near the centre of the Exon 2 amplicon, the two SURVEYOR Nuclease cleavage products have very similar migration times and form a single DNA band. 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 5% with SURVEYOR Nuclease + Lonza FlashGel System.

### Limit of Detection Sequencing Results for K-RAS Exon 2 G12D 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 *Exon 2* 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 Lonza FlashGel System. **NOTE:** to achieve a 90% Wild-Type, 10% Mutant, a blend of 80% PN 710141 and 20% PN 710144 is prepared. Because the G13D mutation is located near the centre of the Exon 2 amplicon, the two SURVEYOR Nuclease cleavage products have very similar migration times and form a single DNA band. 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 5% with SURVEYOR Nuclease + Lonza FlashGel System.

#### 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 (10% 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 5% of wild-type is realistic, this is dependent upon the quality of a given gel. Poor quality genomic DNA or PCR reactions or a sub-optimally gel electrophoresis set-up can all result poor gel resolution or high background and make discrimination of minor bands more challenging.

Use 5% mutation controls, as described in the **Gel Image Quality** section of this User Guide in order to ascertain if the required sensitivity is being achieved.

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

## Instructions For Use of the SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* Gel Electrophoresis

- Sequence the sample to confirm the presence of a mutation. Low percentage mutation loads will be difficult to confirm by DNA sequencing.
- 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**.

## 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** will ensure that the extracted DNA is suitable for use in this kit.

This kit has been validated for research use with formalin-fixed paraffin-embedded colorectal cancer tumor biopsy samples. It has not been validated for research 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 **Appendix - Troubleshooting Guide** 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.

## Literature References

1. **Mutation detection using SURVEYOR nuclease.** Qiu P, Shandilya H, D'Alessio JM, O'Connor K, Durocher J, Gerard GF. *BioTechniques* 36, 702-707. (2004).
2. Gerard, G.F. and Shi, Y. unpublished observations (2009).
3. **Improved identification of von Hippel-Lindau gene alterations in clear cell renal tumors.** Nickerson ML, Jaeger E, Shi Y, Durocher JA, Mahurkar S, Zaridze D, Matveev V, Janout V, Kollarova H, Bencko V, Navratilova M, Szeszenia-Dabrowska N, Mates D, Mukeria A, Holcatova I, Schmidt LS, Toro JR, Karami S, Hung R, Gerard GF, Linehan WM, Merino M, Zbar B, Boffetta P, Brennan P, Rothman N, Chow WH, Waldman FM, Moore LE. *Clin Cancer Res.* 14, 4726-34 (2008).
4. **Mutations in the LKB1 tumour suppressor are frequently detected in tumours from Caucasian but not Asian lung cancer patients.** Koivunen JP, Kim J, Lee J, Rogers AM, Park JO, Zhao X, Naoki K, Okamoto I, Nakagawa K, Yeap BY, Meyerson M, Wong KK, Richards WG, Sugarbaker DJ, Johnson BE, Jänne PA. *Br. J. Cancer.* 99, 245-52 (2008).
5. **Development of a simple and highly sensitive mutation screening system by enzyme mismatch cleavage with optimized conditions for standard laboratories.** Tsuji T, Niida Y. *Electrophoresis* 29, 1473-83 (2008).
6. **TP53 mutations and survival in squamous-cell carcinoma of the head and neck.** Poeta ML, Manola J, Goldwasser MA, Forastiere A, Benoit N, Califano JA, Ridge JA, Goodwin J, Kenady D, Saunders J, Westra W, Sidransky D, Koch WM. *N. Engl. J. Med.* 357,:2552-61 (2007).
7. **SURVEYOR nuclease-based detection of p53 gene mutations in haematological malignancy.** Mitani N, Niwa Y, Okamoto Y. *Ann. Clin. Biochem. Nov;44(Pt 6):557-9 (2007).*

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8. **KIT-negative undifferentiated endometrial sarcoma with the amplified epidermal growth factor receptor gene showing a temporary response to Imatinib mesylate.** Mitsuhashi T, Nakayama M, Sakurai S, Fujimura M, Shimizu Y, Ban S, Ogawa F, Hirose T, Ishihara O, Shimizu M. *Ann. Diagn. Pathol.* 11, 49-54. (2007).
9. **Allelic dilution obscures detection of a biologically significant resistance mutation in EGFR -amplified lung cancer.** Engelman JA, Mukohara T, Zejnullahu K, Lifshits E, Borrás AM, Gale CM, Naumov GN, Yeap BY, Jarrell E, Sun J, Tracy S, Zhao X, Heymach JV, Johnson BE, Cantley LC, Jänne PA. *J. Clin. Invest.* 116, 2695-2706. (2006).
10. **Erlotinib for frontline treatment of advanced non-small cell lung cancer: a phase II study.** Giaccone G, Gallegos Ruiz M, Le Chevalier T, Thatcher N, Smit E, Rodriguez JA, Jänne P, Oulid-Aissa D, Soria JC. *Clin. Cancer Res.* 12, 6049-6055. (2006).
11. **Genetic predictors of adverse radiotherapy effects: the Gene-PARE project.** Ho AY, Atencio DP, Peters S, Stock RG, Formenti SC, Cesaretti JA, Green S, Haffty B, Drumea K, Leitzin L, Kuten A, Azria D, Ozsahin M, Overgaard J, Andreassen CN, Trop CS, Park J, Rosenstein BS. *Int. J. Radiat. Oncol. Biol. Phys.* 65, 646-655. (2006).
12. **Response and resistance in a non-small-cell lung cancer patient with an epidermal growth factor receptor mutation and leptomeningeal metastases treated with high-dose gefitinib.** Jackman DM, Holmes AJ, Lindeman N, Wen PY, Kesari S, Borrás AM, Bailey C, de Jong F, Jänne PA, Johnson BE. *J. Clin. Oncol.* 24, 4517-4520. (2006).
13. **Exon 19 deletion mutations of epidermal growth factor receptor are associated with prolonged survival in non-small cell lung cancer patients treated with Gefitinib or Erlotinib.** Jackman DM, Yeap BY, Sequist LV, Lindeman N, Holmes AJ, Joshi VA, Bell DW, Huberman MS, Halmos B, Rabin MS, Haber DA, Lynch TJ, Meyerson M, Johnson BE, Janne PA. *Clin. Cancer Res.* 12, 3908-3914. (2006).
14. **A rapid and sensitive enzymatic method for epidermal growth factor receptor mutation screening.** Janne PA, Borrás AM, Kuang Y, Rogers AM, Joshi VA, Liyanage H, Lindeman N, Lee JC, Halmos B, Maher EA, Distel RJ, Meyerson M, Johnson BE. *Clin. Cancer Res.* 12, 751-758. (2006).
15. **Effect of epidermal growth factor receptor tyrosine kinase domain mutations on the outcome of patients with non-small cell lung cancer treated with epidermal growth factor receptor tyrosine kinase inhibitors.** Jänne PA, Johnson BE. *Clin. Cancer Res.* 12, 4416s-4420s. (2006).
16. **Mutation analysis of hCDC4 in AML cells identifies a new intronic polymorphism.** Nowak D, Mossner M, Baldus CD, Hopfer O, Hofmann WK. *Int. J. Med. Sci.* 3, 148-151. (2006).
17. **Activity of the tyrosine kinase inhibitor PKC412 in a patient with mast cell leukemia with the D816V KIT mutation.** Gotlib J, Berube C, Growney JD, Chen CC, George TI, Williams C, Kajiguchi T, Ruan J, Lilleberg SL, Durocher JA, Lichy JH, Wang Y, Cohen PS, Arber DA, Heinrich MC, Neckers L, Galli SJ, Gilliland DG, Coutre SE. *Blood* 106, 2865-2870. (2005).
18. **Genetic variance detection using SURVEYOR Nuclease.** Gerard GF, Shandilya H, Qiu P, Shi Y, Lo J. In *Genetic Variance Detection - Technologies for Pharmacogenomics* (ed. Hecker KH) DNA Press, Eagleville, PA, pp. 95 – 129. (2006).

## Appendix

### Troubleshooting Guide

Effective use of the SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* 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** after reading and understanding the section **Step-by-Step Instructions**. 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 *Exon 2* 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/ $\mu$ L 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  $A_{260}$  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/ $\mu$ L) 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 ensure that the expected digestion products are obtained, free from non-specific PCR amplicon products (see **Examples of Results, Figures 3-4**). Examine each amplified DNA product before digestion by gel electrophoresis 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**) in order to maximize the efficiency of heteroduplex formation. Heteroduplexes are very inefficiently formed during standard PCR reactions.

**Note:** band intensity obtained is generally high enough to detect mutations present at a low percentage of the total DNA template; it is possible to detect 5% mutant DNA. **Figures 5 and 6** show detection of K-RAS Exon 2 codon 12 and 13 mutations present (9.5% heteroduplex) by agarose gel electrophoresis. **Figures 3-4** show the digestion products generated with homoduplex and heteroduplex K-RAS Positive Control DNAs (included in this kit) by agarose gel electrophoresis. The mutation-specific cleavage products are clearly seen as bands migrated 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: <ol style="list-style-type: none"> <li>1. Make sure the DNA concentration is in the range of &gt;25 ng/μL.</li> <li>2. Repeat the hybridization step, taking care to follow the Hybridization procedure.</li> </ol>
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 digestion<sup>18</sup>. 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 bands or smearing, comparing control digests of homoduplexes with sample digests will allow these to be recognized.

**Problem 5 – SURVEYOR Nuclease digestion peaks in negative controls**

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

**Problem 6- Gel image is not clear**

<b>POSSIBLE CAUSE</b>	<b>SOLUTION</b>
Poor electrical contact with gel	Assure that the gel and/or its cassette are firmly seated
Gel was run too fast	Follow times in this manual for the gel
Incorrect gel concentration was used	Use the % agarose gels recommended in this User Guide
Voltage was too high	Follow the voltages recommended in this User Guide as opposed to those specified by the manufacturers
Transilluminator settings	Check that the proper filter and aperture settings are being used.
Photograph of gel image is indistinct	Follow the recommendations for a suitable camera system and test using a 5% mutant plasmid sample. Increase exposure time.

# Instructions For Use of the SURVEYOR Scan K-RAS Mutation Detection Kit *Exon 2* Gel Electrophoresis

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### ISO 9001:2008 Certification

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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