

# SURVEYOR® Check-It Kit Clone Sequence Validation

**Detects all types of point mutations and small insertions/deletions introduced as PCR artifacts in cloned DNA or by site-directed mutagenesis**

The SURVEYOR Check-It Kit for Clone Sequence Validation has been specifically designed to analyze cloned DNA to detect mutations and errors which may have been introduced into insert sequences during the PCR amplification and cloning processes, or to verify the presence of desired mutations deliberately introduced by site-directed mutagenesis. The kits include SURVEYOR Nuclease, a mismatch-specific plant endonuclease that cleaves the cleaves DNA heteroduplexes at all the possible mismatch sites.<sup>1-4</sup> The DNAs of prospective clones are PCR amplified directly from colonies. After annealing to reference DNA and treatment of the duplexes with SURVEYOR Nuclease, the cleavage fragments are separated by standard agarose gel electrophoresis and visualized by staining with ethidium bromide or SYBR® Gold.

## Mutation Detection in Four Easy Steps



## Applications and Benefits

- Identifies error-free cDNA or genomic DNA clones that have been generated using PCR-based cloning methods
- Eliminates the need to purify clone DNA
- Verifies that clones modified specifically by site-directed mutagenesis contain the desired mutation and lack undesirable mutations
- Eliminates the need to isolate and sequence DNA from multiple colonies

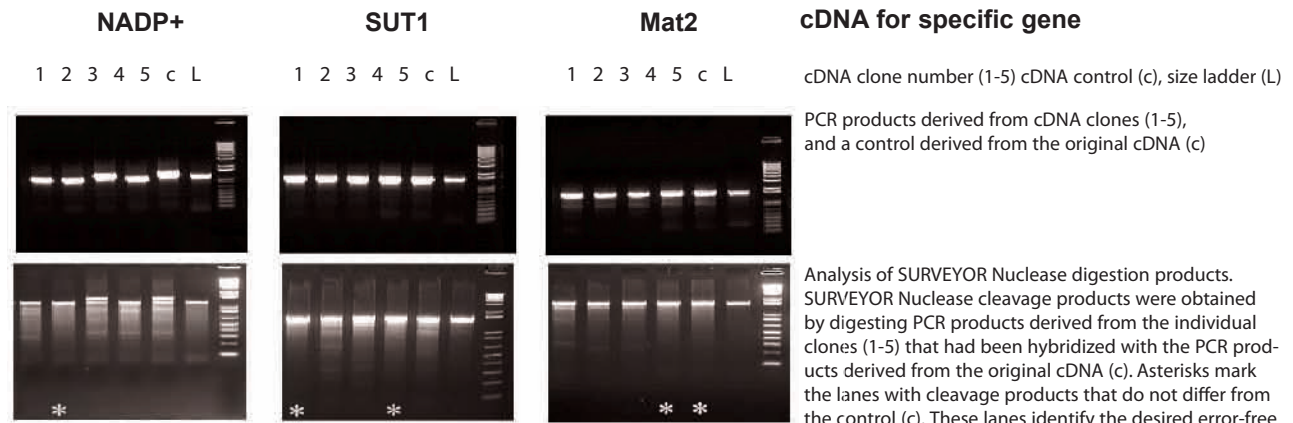
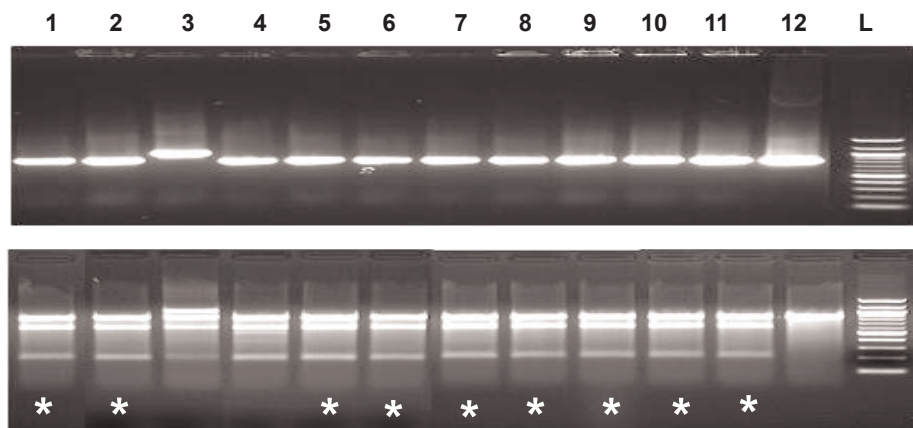


Figure 1. Identification of clones containing error-free inserts

PCR-based cloning and site-directed mutagenesis are established methods requiring confirmation that the cloned insert possesses the desired sequence. This confirmation and identification step usually required direct DNA sequencing of the insert DNA in multiple clones. When you use SURVEYOR Check-It Kit and analyze the inserts' PCR products and the SURVEYOR Nuclease digestion products by agarose gel electrophoresis as shown in Figure 1, you no longer need to sequence every clone.

Colonies derived from site-directed mutagenesis experiments can also be easily checked to make sure that unwanted mutations are absent, and that desired mutations are actually present.



Analysis of PCR products of individual colonies bearing a potential mutant plasmid (lanes 1 to 11) obtained by site-directed mutagenesis, and the wild-type plasmid pQIS155 as a control (lane 12).

“Mutant” PCR products shown in the upper panel were annealed with amplified wild-type DNA derived from plasmid pQIS155 (Reference DNA), and digested with SURVEYOR Nuclease. Lanes of homogeneous clones with the desired mutation are marked with asterisks.

Figure 2. Analysis of clones modified by site-directed mutagenesis.

## SURVEYOR Mutation Detection Kit Components – All Optimized to Work Together

- SURVEYOR Nuclease (for 100 reactions)
- Stop Solution
- Control Plasmids and Primers

### Products

**SURVEYOR Check-It Kit**

**Quantity**  
100 reactions

**Catalog No.**  
706040

### Shipping and Storage

SURVEYOR products are shipped frozen. Store product at -20° C in a non-frost-free freezer. Enzyme is guaranteed for a period of 6 months if stored as directed.

### Quality Control

Every component has met Transgenomic quality control standards. Refer to the *Certificate of Analysis* for details.

## Selected References

1. **Rapid identification of unknown heteroplasmic mutations across the entire human mitochondrial genome with mismatch-specific Surveyor Nuclease** Bannwarth, S., Procaccio, V., Paquis-Flucklinger, V., *Nature Protocols* VOL.1, NO.4 2037-2047. (2006).
2. **Allelic dilution obscures detection of a biologically significant resistance mutation in EGFR-amplified lung cancer.** Engelman, J.A., Mukohara, T., Zejnullahu, K., Lifshits, E., Borras, A.M., Gale, C.M., Naumov, G.N., Yeap, B.Y., Jarrell, E., Sun, J., Tracy, S., Zhao, X., Heymach, J.V., Johnson, B.E., Cantley, L.C., Janne, P.A., *J. Clin. Invest.* 116, 2695-2706. (2006).
3. **The JAK2 V617F mutation occurs in hematopoietic stem cells in polycythemia vera and predisposes toward erythroid differentiation.** Jamieson, C.H., Gotlib, J., Durocher, J.A., Chao, M.P., Mariappan, M.R., Lay, M., Jones, C., Zehnder, J.L., Lilleberg, S.L., Weissman, I.L. *Proc. Natl. Acad. Sci.* 103, 6224-6229 (2006).
4. **A rapid and sensitive enzymatic method for epidermal growth factor receptor mutation screening.** Janne, P.A., Borras, A.M., Kuang, Y., Rogers, A.M., Joshi, V.A., Liyanage, H., Lindeman, N., Lee, J.C., Halmos, B., Maher, E.A., Distel, R.J., Meyerson, M., Johnson, B.E. *Clin. Cancer Res.* 12, 751-758. (2006).

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