

## How Neogen's Test Kits Work

### Sandwich DNA Hybridization Assays

(*GENE-TRAK pathogen test kits in dipstick and microwell formats, and GeneQuence test systems in microwell format, excluding GeneQuence E. coli O157:H7*)

Each test kit contains capture and detector DNA probes specific to ribosomal RNA (rRNA) of the target organism and a coated solid phase (dipstick or microwell).

First, a portion of the enrichment culture is placed into a test tube. A lysis reagent is added, which disrupts the cell and releases the nucleic acid target molecules. A portion of the lysed sample is then transferred to a microwell and the probe reagents are added.

(In the dipstick format assay, the probe reagents are added to the tube with the lysed sample, followed by introduction of the coated dipstick to the tube.)

The probe reagents consist of: 1) an oligonucleotide capture probe specific to rRNA sequences of the target organism and labeled at the 3' end with polydeoxyadenylic acid (poly dA); and 2) an oligonucleotide detector probe also specific to rRNA sequences of the target organism and labeled at the 5' end with the enzyme horseradish peroxidase (HRP).

The hybridization reaction is then allowed to proceed for one hour. If target rRNA is present in the sample, both probes will hybridize to their complementary sequences on the target molecule. The resulting complex is captured onto the solid phase coated with polydeoxythymidylic acid (poly dT), which is complementary to the poly dA portion of the capture probe. Unbound probe is then washed away, and a substrate of HRP is added.

Following a short incubation, blue color indicates the presence of hybridized detector probe in the complex and thus the presence of rRNA from the target organism. Results are determined spectrophotometrically at 450 nm. An absorbance value in excess of a predetermined threshold indicates a positive test result.

