



THE POWER OF MOLECULAR DIAGNOSTICS IN THE PALM OF YOUR HAND™

ASSAY PRINCIPLES

Veriflow™ *Green Fluorescent Protein (GFP)* is a molecular based assay for the presumptive and qualitative detection of *GFP* in SLR 2954 *Salmonella abaeetuba GFP*, 35150 *E. coli O157:H7 GFP*, and SLR 3032 *Listeria monocytogenes GFP*. The assay utilizes a PCR detection method coupled with a rapid, visual, flow-based assay that develops in 3 minutes post PCR amplification and requires only 18-24 hours of incubation for maximum sensitivity. The Veriflow™ *GFP* system eliminates the need for gel electrophoresis or fluorophore based detection of target amplifications, and does not require complex data analysis. Veriflow™ *GFP* provides the specificity and sensitivity of PCR based amplification in a cost-efficient and easy-to-use format.

INTENDED USER

The Veriflow™ *GFP* system is intended for use by personnel familiar with basic sample collection and preparation techniques associated with foodborne pathogen detection. Veriflow™ *GFP* is specifically designed to be easy-to-use and eliminates the need for advanced training in molecular biology.

Invisible Sentinel™ and Veriflow™ are trademarks of Invisible Sentinel, Inc. of Philadelphia, PA. Stomacher® is a registered trademark of Tekmar, Inc., Cincinnati, OH. Spec-Sponge® and Whirl-Pak® are registered trademarks of eNasco of Fort Atkinson, WI. 3M™ Enviro Swab is a trademark of 3M, Inc. of Minneapolis, MN. U.S. Patent No. 8,183,059 and other patents pending. Purchase and use of this product is subject to Invisible Sentinel's Terms and Conditions of Sale located at <http://www.invisiblesentinel.com>.



Phone 215.966.6118 | Fax 215.386.3970

Email info@invisiblesentinel.com | www.invisiblesentinel.com

MATERIALS PROVIDED

1. IS *GFP* PCR Reagent - Cat. No. IS506
2. IS Buffer B - Cat. No. IS0702
3. IS Veriflow™ *GFP* Assay Cassette - Cat. No. IS0109
4. 1.5 mL Sample Boil Tubes - Cat. No. IS0902

MATERIALS NEEDED for UHT SKIM MILK SAMPLES

1. 24 oz. (710 mL) Incubation Bags (Milk Samples).
 - a. e.g. Nasco Cat. No. B01401WA or equivalent.

MATERIALS NEEDED for DOG FOOD SAMPLES

1. 24 oz. (710 mL) or 92 oz. (2721 mL) incubation bags (Dog Food Samples).
 - a. e.g. Nasco Cat. No. B01348WA or Nasco Cat. No. B01488 or equivalent.

MATERIALS NEEDED for ENVIRONMENTAL SAMPLES

1. Dry or pre-moistened sampling sponges OR swabs
 - a. e.g. Nasco Speci-Sponge® Cat. No. B01245 or equivalent OR 3M™ Enviro Swab Cat. No. ENVSWB25D or equivalent.
 - b. Sponge should not exceed 10 mL, and Swab should not exceed 2 mL of hydration with Dey-Engley broth.
2. Dey-Engley broth (if necessary)
 - a. e.g. BD Cat. No. 281910 or equivalent
3. 18 oz (532 ml) incubation bag for sponge samples
 - a. e.g. Nasco Whirl-Pak® Cat. No. B01245 or equivalent
4. 2 oz (58 ml) incubation bag for swab samples
 - a. e.g. Nasco Whirl-Pak® Cat. No. B01009WA or equivalent

MATERIALS NEEDED for ALL ENRICHMENT TYPES

1. Incubator that provides continuous and stable temperatures of 35°C ± 1°C
2. Hot plate/heat source for boiling water bath
3. Heat tolerant beaker (optional for boiling)
4. PCR Thermocycler
5. Pipettes and tips for 5, 200, and 1000 µl volumes
6. Glassware and autoclave for media prep
7. Racks for culture bags and 1.5 mL tubes
8. dH₂O
9. Scale for weighing of sample and media
10. Freezer capable of maintaining -20°C

STORAGE OF MATERIALS

The IS *GFP* kit components, including cassettes, plastics, growth media and buffers should be stored at room temperature (20-25°C). The Veriflow™ *GFP* test PCR reagents should be stored at -20°C.

PRECAUTIONS

1. *Salmonella abaeetuba GFP*, *E. coli O157:H7 GFP*, and *Listeria monocytogenes GFP* are human pathogens. All samples collected for use with the Veriflow™ *GFP* Assay should be handled with care.
2. Assay users should observe standard microbiological practices and safety precautions when performing this assay.
3. Do not use Veriflow™ *GFP* Assay cassettes past indicated expiration date.
4. Deviations from the assay protocol may impact overall test performance.

SECTION I: SAMPLING AND ENRICHMENT for DAIRY SAMPLES

1. Transfer 225 mL Lactose Broth (BD, 214908) (*Salmonella abaeetuba*

GFP, E. coli O157:H7 GFP or IS Listeria Broth (InvSent, IS0302) (*Listeria monocytogenes GFP*) to a 24 oz. (710 mL) incubation bag.

2. Transfer 25 mL sample directly to bag from step 1.
3. Agitate bag to evenly distribute sample.
4. Place bag into 35°C incubator, in rack, for 18-24 hours: *Salmonella abaeetuba GFP, E. coli O157:H7 GFP* or 24-28 hours: *Listeria monocytogenes GFP*.

SECTION II: SAMPLING and ENRICHMENT for 25 GRAM DOG FOOD SAMPLES

1. Weigh out 25 gram sample.
2. Transfer 25 gram sample to 24 oz. (710mL) incubation bag.
3. Transfer 225 mL enrichment media to incubation bag containing sample.
4. Stomach to break down sample.
5. Place bag into 35°C incubator, in rack, for 18-24 hours: *Salmonella abaeetuba GFP, E. coli O157:H7 GFP* or 24-28 hours: *Listeria monocytogenes GFP*.

SECTION III: SAMPLING and ENRICHMENT for 325 GRAM DOG FOOD SAMPLES

1. Weigh out 325 gram sample.
2. Transfer 325 gram sample to 92 oz. (2721 mL) incubation bag.
3. Transfer 1625 mL enrichment media to incubation bag containing sample.
4. Stomach to break down sample.
5. Place bag into 35°C incubator, in rack, for 18-24 hours: *Salmonella abaeetuba GFP, E. coli O157:H7 GFP* or 24-28 hours: *Listeria monocytogenes GFP*.

SECTION IV: SPONGE SURFACE SAMPLING AND ENRICHMENT

1. If sponges are not pre-moistened, pipette 10 mL of Dey-Engley Neutralization broth to each sponge placed in an 18 oz. (532 mL) culture bag.
2. Squeeze excess moisture from sponge and remove from culture bag.
3. Sample surface area of interest using sponge for 30 seconds.
4. Transfer sponge back to culture bag.
5. Transfer 200 mL enrichment media into bag containing sponge used for sampling, seal, and briefly massage sponge.
6. Place bag into 35°C incubator, in rack, for 18-24 hours: *Salmonella abaeetuba GFP, E. coli O157:H7 GFP* or 24-28 hours: *Listeria monocytogenes GFP*.

SECTION V: SWAB SURFACE SAMPLING AND ENRICHMENT

1. If swabs are not pre-moistened, pipette 2 mL of Dey-Engley Neutralization broth to each swab placed in a 2.0 oz. (58mL) culture bag.
2. Squeeze excess moisture from swab and remove from culture bag.
3. Sample surface area of interest using swab for 30 seconds.
4. Transfer swab back to culture bag.
5. Transfer 20 mL enrichment media into bag containing swab used for sampling, seal, and briefly massage swab.
6. Place bag into 35°C incubator, in rack, for 18-24 hours: *Salmonella abaeetuba GFP, E. coli O157:H7 GFP* or 24-28 hours: *Listeria monocytogenes GFP*.

SAMPLE PREP and PCR

1. Place provided 1.5 mL *GFP* Sampling tubes in rack (1 for each sample to be tested).
2. Remove culture enrichment bag from incubator and agitate to suspend any settled contents.
3. Pipette 1 mL of enriched culture (*Salmonella abaeetetuba GFP*, *E. coli O157:H7 GFP*) or 500 μ L enriched culture + 500 μ L dH₂O (*Listeria monocytogenes GFP*) to prepared 1.5mL tube from step 1 above, seal, and invert to mix contents.
4. Boil sample from step 3 above in water bath or heating block for 10 minutes and allow to equilibrate to room temperature (20-25°C), 10-20 minutes.
 - a. Note: samples can be stored sealed at -20°C, pre or post boil, for 1 week, prior to step 5 below.
5. Transfer 5 μ L of liquid from cooled boiled sample from step 4 above to thawed PCR reagent tube for each sample.
 - a. Note: Open PCR tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
6. Cycle sample PCR tube in thermocycler with Veriflow™ *GFP* program.

FLOWTHROUGH CASSETTE SAMPLE ANALYSIS

1. Remove tubes from thermocycler and add 4 drops of Buffer B directly to each PCR tube.
2. Transfer entire contents (200 μ L) of PCR tube directly to Veriflow™ *GFP* Assay cassette sample window with pipette. A separate Veriflow™ *GFP* Assay cassette must be used for each PCR tube.
3. Allow test to develop for 2 minutes.
4. Add 4 drops of Buffer B directly to each Veriflow™ *GFP* Assay cassette sample window.
5. Allow test to develop for 1 minute.
6. Retract switch and record results.
 - a. The appearance of one red line (control) in the cassette window indicates a negative result.
 - b. The appearance of two red lines (control and test) in the cassette window indicates a positive result.

SAMPLING for COLONY PCR

1. Add 500 mL sterile dH₂O to a 1.5 mL tube.
2. Pick a well-isolated single colony from plate using a 200 mL pipette.
3. Resuspend the colony in the 1.5 ml tube containing sterile dH₂O and vortex to mix contents.
4. Transfer 5 mL from the tube into a PCR tube.

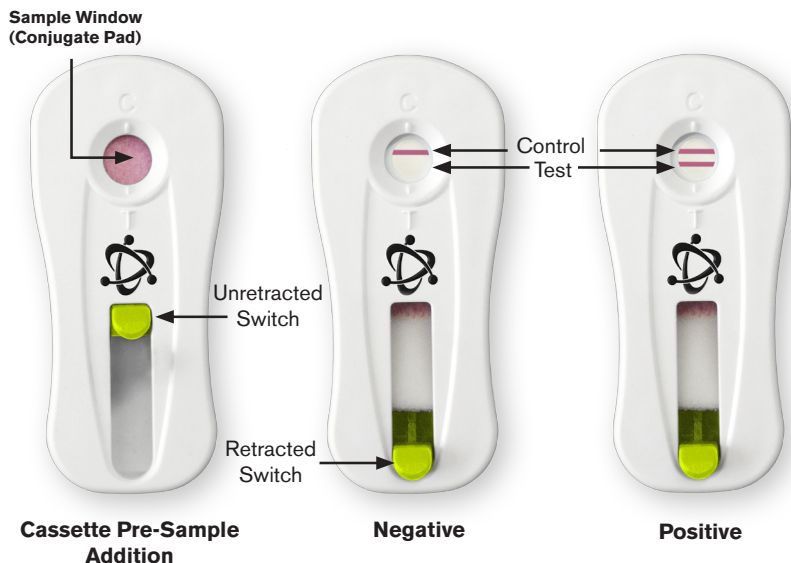
CUSTOMER SERVICE

Invisible Sentinel customer service and technical assistance can be reached between 9AM and 5PM Eastern time by calling 215-966-6118 and asking for an Invisible Sentinel sales or technical representative. Training on this product and all Invisible Sentinel test kits is available.

MSDS INFORMATION AVAILABLE

Material Safety Data Sheets (MSDS) are available for this test kit and all of Invisible Sentinel's Food test kits by calling Invisible Sentinel at 215-966-6118.

APPENDIX 1: RESULTS INTERPRETATION



The control line, as indicated by the letter C on the test cassette, should always develop. The test line, as indicated by the letter T on the cassette, will only develop in the event of a positive sample for *Green Fluorescent Protein*. If the control line fails to develop, the test is invalid, and will need to be repeated.

APPENDIX 2: DISPOSAL

Invisible Sentinel devices are for single use only. Decontaminate all surfaces, media and reagents and discard in accordance with local, state, and federal regulations.