

LinkSēq™ Wipe Test Kit Short Protocol

REF 9850R-SL, 9850R-SR

Kit Components (stored at -35 to -15°C)

- 12 Strips containing dried reagents – RED Caps/RED Indicator Line
 - One test requires 3 strips. Each test can test 6 surface swab samples.
- 1 vial of WT Buffer
- 1 vial of WT Polymerase
- 1 vial of LWT Enzyme
- 1 vial of Control DNA

The strip is configured as follows:

| | |
|---|------------|
| A | SSS 1 |
| B | SSS 2 |
| C | SSS 3 |
| D | SSS 4 |
| E | SSS 5 |
| F | SSS 6 |
| G | DNA (+) |
| H | NCS (-) |

Figure 1: 8-Well Strip Layout

Note: 3 strips are required to perform one wipe test (6 surface swab samples).

Table 1: Strips Configuration

| Strips Location | Sample Source |
|-----------------|---------------------------------|
| A-F | Surface Swab Samples – SSS |
| G | Control DNA – DNA (+) |
| H | Negative Control Swab – NCS (-) |

Real-Time PCR Instrument Set-Up

Refer to the LinkSeq Wipe Test Application Note for instrument set-up instructions.

Notes:

1. **Minimize the time between sample addition and initiation of thermal cycling.**
2. **Do not allow the pipette tip to come in contact with the bottom of the well.**
3. **Transfer any extra sample mix from the tip back into the tube to conserve supply (some repeat pipettors have large dead volumes).**
4. **Make sure the tray is completely sealed. Any openings in the seal can cause evaporation during amplification.**
5. **Use molecular biology grade water.**

Sample Setup Protocol

Sample Preparation

In preparation for sample collection, assemble the following materials:

- 7 Sterile Applicator Swabs
- 7 Sterile 2.0 mL Tubes
- 55°C Water Bath or Heat Block

Selecting Areas for Swabbing

Up to 6 surface swab samples can be tested with 3 strips.

Sample Collection Protocol

1. Track sample information on the provided *Strips Reference* for input into SureTyper™.
2. In a designated clean area (e.g. PCR workstation), label 2.0 mL tubes for each of the 6 sample locations plus the negative control swab (NCS) sample (7 tubes total).
3. Using filtered pipette tips, aliquot 500 µL molecular grade water into each tube.
4. Insert a sterile applicator swab in each tube for 1 minute to allow for absorption.
5. Wipe the sample location with the moistened applicator swab, and place it back into the original tube.
6. Snap off the plastic end of the applicator so that the swab fits in the tube and allows for proper closure of the tube. Cap the tube securely. Vortex briefly.
7. Prepare the negative control swab sample by snapping off a clean applicator swab into the seventh (NCS) tube and cap the tube securely. Vortex briefly.
8. Incubate all tubes in a 55° C water bath or heat block for 1 hour.
9. Centrifuge tubes for 1 minute at the highest speed in a microcentrifuge (~14,000 x g).
10. Carefully remove and discard the applicator swab from each tube.
11. Centrifuge tubes for 1 minute at the highest speed in a microcentrifuge (~14,000 x g).

PCR Set-Up Protocol

1. Thaw the WT Buffer at room temperature.
2. Remove the wipe test strips from the freezer and place at room temperature. (3 strips are required for one wipe test of 6 surface swab samples).
3. Quickly spin down the strips at 500-2,000 x g for 30-60 seconds.
4. Vortex to mix and pulse spin WT Buffer to ensure contents are collected at the bottom of the tube.
5. Remove the WT Polymerase and LWT Enzyme from the -20 °C freezer and place on ice.

6. Label 2.0 mL tubes with “A,” “Ag,” and “B.”
7. Prepare the following buffer tubes as follows:

Buffer A

Standard Reactions

| | | |
|-----------------|----------------|--------------------|
| Buffer A | WT Buffer (μL) | WT Polymerase (μL) |
| | 192 | 8 |

No Water Added

Note: Buffer Ag and Buffer B require an aliquot of Buffer A (after WT Polymerase addition). **Return WT Buffer and WT Polymerase tubes to the freezer.**

Buffer Ag

Standard Reactions + Control DNA

| | | |
|------------------|---------------|------------------|
| Buffer Ag | Buffer A (μL) | Control DNA (μL) |
| | 60 | 12 |

No Water Added

Buffer B

Standard Reactions + LWT Enzyme

| | | |
|-----------------|---------------|-----------------|
| Buffer B | Buffer A (μL) | LWT Enzyme (μL) |
| | 65 | 1.5 |

No Water Added

8. Remove the strip caps and discard.
9. Label the strip tabs with “A,” “Ag,” and “B.”

10. Set up the sample strips as described below (Refer to the *Reagent Distribution Table*):
 - a. Add 5 µL of Buffer A to Strip A positions A-H.
 - b. Add 5 µL of Buffer Ag to Strip Ag positions A-H.
 - c. Add 5 µL of Buffer B to Strip B position A-H.
 - d. Add 5 µL Surface Swab Sample(s) to the indicated sample positions (Strip A positions A-F, Strip Ag positions A-F, and Strip B positions A-F).
 - e. Add 5 µL of molecular biology grade water to position (+) (Strip A position G, Strip Ag position G, and Strip B position G).
 - f. Add 5 µL of the Negative Control (NC) Swab to position (-) (Strip A position H, Strip Ag position H, and Strip B position H).
 - g. Add 1 µL of Control DNA to Strip A position G and Strip B position G.

Note: Strip A position G and Strip B position G will have a final volume of 11 µL.
11. Seal the strips by placing the PCR Optical Strip Caps on to each appropriate strip.

Note: Make sure the strips are completely sealed. Any openings can cause evaporation during amplification.
12. Spin the strips at 500-2,000 x g for 30-60 seconds and proceed to Step 13 immediately.
13. Set the three strips as described in Figure 3: Real-Time PCR Instrument Strip Loading below.
14. Record the selected real-time PCR position columns on the *Strips Reference* sheet.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|----------|-----------|----------|----------|-----------|----------|----------|-----------|----------|----------|-----------|----------|
| A | | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | Buffer A | Buffer Ag | Buffer B | Buffer A | Buffer Ag | Buffer B | Buffer A | Buffer Ag | Buffer B | Buffer A | Buffer Ag | Buffer B |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |
| | Option 1 | | | Option 2 | | | Option 3 | | | Option 4 | | |

Figure 3: Real-Time PCR Instrument Strip Loading

Set the three strips into the real-time PCR instrument in one of the following sets of columns: 1-3, 4-6, 7-9, or 10-12. The Buffer A strip should be loaded into the lowest number, followed by the Buffer Ag strip loaded into the middle number, and the Buffer B strip loaded into the highest number of the set of 3. (See Table 2 below for more information regarding loading). Begin amplification.

Note: All positions of the instrument block may be loaded in a single read for a total of 12 strips.

15. Start the program described in **Real-Time PCR Instrument Set-Up** above.
16. After amplification and dissociation are completed, export the data from the real-time PCR instrument.

Reagent Distribution Table

Set up the sample strips as described below:

- Add 5 μ L of Buffer A to Strip A positions A-H.
- Add 5 μ L of Buffer Ag to Strip Ag positions A-H.
- Add 5 μ L of Buffer B to Strip B position A-H.
- Add 5 μ L Surface Swab Sample(s) to the indicated sample positions (Strip A positions A-F, Strip Ag positions A-F, and Strip B positions A-F).
- Add 5 μ L of molecular biology grade water to position (+) (Strip A position G, Strip Ag position G, and Strip B position G).
- Add 5 μ L of the Negative Control (NC) Swab to position (-) (Strip A position H, Strip Ag position H, and Strip B position H).
- Add 1 μ L of Control DNA to Strip A position G and Strip B position G.

Note: Strip A position G and Strip B position G will have a final volume of 11 μ L.

| | A | Ag | B |
|---|--|---|--|
| A | 5 μ L Sample 1 5 μ L Buffer A | 5 μ L Sample 1 5 μ L Buffer Ag | 5 μ L Sample 1 5 μ L Buffer B |
| B | 5 μ L Sample 2 5 μ L Buffer A | 5 μ L Sample 2 5 μ L Buffer Ag | 5 μ L Sample 2 5 μ L Buffer B |
| C | 5 μ L Sample 3 5 μ L Buffer A | 5 μ L Sample 3 5 μ L Buffer Ag | 5 μ L Sample 3 5 μ L Buffer B |
| D | 5 μ L Sample 4 5 μ L Buffer A | 5 μ L Sample 4 5 μ L Buffer Ag | 5 μ L Sample 4 5 μ L Buffer B |
| E | 5 μ L Sample 5 5 μ L Buffer A | 5 μ L Sample 5 5 μ L Buffer Ag | 5 μ L Sample 5 5 μ L Buffer B |
| F | 5 μ L Sample 6 5 μ L Buffer A | 5 μ L Sample 6 5 μ L Buffer Ag | 5 μ L Sample 6 5 μ L Buffer B |
| G | 5 μ L H2O 1 μ L Control DNA 5 μ L Buffer A | 5 μ L H2O 5 μ L Buffer Ag | 5 μ L H2O 1 μ L Control DNA 5 μ L Buffer B |
| H | 5 μ L NC Swab 5 μ L Buffer A | 5 μ L NC Swab 5 μ L Buffer Ag | 5 μ L NC Swab 5 μ L Buffer B |

Buffer A = Standard

Buffer Ag = Standard + gDNA

Buffer B = Standard + LWT