LinkSēq™ Wipe Test Kits

REF

Table 1: Product Description

Kit Name	Part No.
LinkSēq Wipe Test 384 Kit	9800R
LinkSēq Wipe Test Kit	9850R-SL
LinkSēq Wipe Test Kit	9850R-SR

For Research Use Only. Not for use in diagnostic procedures.

REAGENTS

A. Identification

The LinkSēq Wipe Test detects laboratory contamination due to trace amounts of PCR amplicons from previous LinkSēq testing or due to genomic DNA (gDNA), both of which may cause false positives in subsequent PCR reactions. The primer set in the reaction wells are designed to amplify the LinkSēq internal control amplicon. If gDNA or amplicon contamination exists, the wipe test primer set will amplify the target sequence.

Each sample is run with 3 different buffer conditions. Buffer A represents standard LinkSēq amplification conditions. Buffer Ag is Buffer A with Control DNA added to the sample. Buffer B is Buffer A with LWT Enzyme added. Prior to PCR cycling, Buffer B sample mixtures are incubated with LWT Enzyme and then inactivated by heat. LWT Enzyme prevents amplification of LinkSēq amplicon; however, LWT Enzyme has no effect on gDNA. The absence and/or presence of peaks in the 3 buffer conditions is used to determine a result for a given sample.

In the LinkSēq Wipe Test, a fluorescent, double-stranded DNA-binding dye (SYBR® Green) is used to identify the presence or absence of amplification products. A real-time PCR instrument is used to detect these products. Raw fluorescence, first derivative (-dF), and temperature data are exported from the real-time PCR instrument and imported into SureTyper™. SureTyper software then plots -dF against temperature to generate a melt-curve for each reaction well. Next, SureTyper (STTPGRX) examines the melt-curves from each well and identifies the amplification peaks as present or absent (peak or no peak). Lastly, SureTyper then analyzes the presence or absence of the peaks in the 3 buffer conditions and displays the wipe test results.

The LinkSēq Wipe Test Kit provides sequence specific primers dried in a PCR Tray for the identification of potential LinkSēq amplicon contamination and genomic DNA contamination.

B. Warning or Caution

1. Regulatory Designation: For Research Use Only—Not for use in diagnostic procedures. Not intended for any animal or human therapeutic use, unless otherwise stated.
2. LinkSēq trays and strips are for single use only. Do not reuse.
3. **Warning**: The WT Buffer components may be harmful by inhalation, ingestion, or skin absorption. Avoid contact with eyes, skin, and clothing. Safe laboratory practices and personal protective equipment should be used at all times during the handling of all product components. Refer to the LinkSēq Safety Data Sheet (SDS) for detailed information.
4. **Warning**: DNA samples extracted from patients, which are not included with this product, should

be treated as potentially hazardous. Safe laboratory practices and personal protective equipment should be used at all times during the handling of DNA samples extracted from patients.

5. **Caution**: The WT Buffer should be stored in the dark when not in use.
6. Refer to the Safety Data Sheet for detailed information.

C. Storage Instructions

- Upon receipt, PCR Trays/Strips, WT Buffer, WT Polymerase, LWT Enzyme, and Control DNA should be stored at -35 to -15°C in a non-frost-free freezer.
- PCR Optical Tray Seals or PCR Optical Strip Caps and Sterile Applicator Swabs should be stored at room temperature 20 to 25°C.
- Use before the Expiration Date.

D. Instability Indications:

- Upon receipt of kit, inspect for physical damage to components such as holes in the seals or leaking vials. Do not use damaged or leaking components.

INSTRUMENT REQUIREMENTS

A. The following instrument set-up is required to run the LinkSēq Wipe Test Kits:

1. Real-Time PCR Instrument with the following specifications:

- 96-well or 384-well block format
- Ability to set the real-time PCR instrument reaction volume to 10 µl per well
- Capable of data output between 65°C and 95°C, with data collection approximately every 0.3°C
- Capable of detecting SYBR Green
- Capable of running the following Real-Time PCR Instrument Profile:

IMPORTANT: The LinkSēq Wipe Test thermal cycling parameters differ from standard LinkSēq thermal cycling parameters. Please use the LinkSēq Wipe Test Template on your instrument when running the LinkSēq Wipe Test. (Refer to Real-Time PCR Instrument Profile: LinkSēq Wipe Test below for LinkSēq Wipe Test thermal cycling parameters).

Real-Time PCR Instrument Profile: LinkSēq Wipe Test (Amplification and Dissociation):

Step 1	50°C for 5 minutes 95°C for 10 minutes
Step 2	36 cycles of: 95°C for 15 seconds 64°C for 60 seconds
Step 3	Add dissociation profile: --Start at 95°C for 15 seconds --Drop to 65°C for 30 seconds[1] --Collect data between 65°C and 95°C --95°C for 15 seconds (end) --Optional: 60°C for 15 seconds[1]

[1]Be sure to set the reaction volume to 10 µl per well

- Capable of exporting dissociation data in text or .csv format.

After the first run, verify that the data collected from the real-time instrument are in the correct temperature range: the data should begin no later than 65°C and not end before 95°C. The software settings on some instruments do not reflect the actual collection temperatures (i.e. some data is cut off). If necessary, adjust the temperature collection settings to ensure the full range is collected.

SPECIMEN COLLECTION AND PREPARATION

Refer to the appropriate kit specific LinkSēq Wipe Test Short Protocol for detailed Sample Preparation instructions.

PROCEDURE

A. **Materials Provided– The quantity of each component varies by product**—Refer to the appropriate kit specific *LinkSēq Wipe Test Short Protocol*.

- PCR Trays (384-well or strips) containing dried reagents
- WT Buffer Vial
- WT Polymerase Vial
- LWT Enzyme Vial
- Control DNA Vial
- Sterile Applicator Swabs
- PCR Optical Tray Seals or PCR Optical Strip Caps

B. Materials Required, But Not Provided

- Sterile 2.0 mL Tubes
- 55°C Water Bath or Heat Block
- Molecular Biology Grade Water
- Pipettes and Compatible Tips: 1-10 µl; 10-200 µl; 100-1000 µl
- Vortex Mixer
- Microcentrifuge
- DNAse, RNAse, and Proteinase-free microcentrifuge and/or PCR tubes
- Real-time PCR Instrument meeting the specifications detailed in Instrument Requirements
- Centrifuge capable of spinning down trays and strips at 500-2,000 x g for 30-60 seconds
- Adhesive seal applicator, e.g. P/N AC-030
- Compression Pad for thermal cycler (as instructed in the instrument manufacturer's instructions for use)

Recommended:

- Dispenser capable of pipetting 10 µl, e.g. repeat pipettor with 0.2 mL tip.

C. Directions for Use

The LinkSēq Wipe Test Short Protocol instructions have slight variations which differ by product.

Refer to the appropriate kit specific LinkSēq Wipe Test Short Protocol for detailed instructions.

RESULTS

A. Data Analysis

A fluorescent dye, SYBR Green, is used to identify the presence or absence of amplification products. SYBR Green fluoresces when bound to amplified, double-stranded DNA. Upon heating the amplified DNA, denaturation causes the SYBR to unbind, which results in decreased fluorescence.

After DNA amplification, samples are analyzed on a real-time PCR instrument. The instrument observes the change in fluorescence measured across a temperature range to generate dissociation data, which is represented as first-derivative (-dF) fluorescence levels for a range of temperature readings.

SureTyper analyzes the dissociation data produced by the real-time PCR instrument and generates a melt-curve profile. The melt-curve is examined within predefined temperature ranges which are based on the

expected values of the amplification product. SureTyper evaluates the melt-curve profile and assigns a "peak" or "no peak" call for each well.

Each well contains the LinkSēq internal control primer. 3 buffers are tested per sample (Buffer A, Buffer Ag, and Buffer B).

SureTyper compares the 3 different buffer reactions tested for each sample and determines if the sample contains contamination.

- A standard well (Buffer A) with a peak combined with an LWT Enzyme well (Buffer B) with no peak indicate amplicon contamination.
- A standard well (Buffer A) with a peak combined with an LWT Enzyme well (Buffer B) with a peak indicate gDNA contamination.
- A standard well (Buffer A) with no peak combined with an LWT Enzyme well (Buffer B) with no peak indicate a clean sample.

Standard Well (Buffer A)		LWT Enzyme Well (Buffer B)		Contamination Type
peak	+	no peak	=	**amplicon**
peak	+	peak	=	**gDNA**
no peak	+	no peak	=	**clean**

Note: Because Buffer Ag contains Control DNA, Buffer Ag reaction wells should always have an amplification peak. If a Buffer Ag reaction well fails to create a peak, this may indicate PCR inhibition or possible set-up problems. The following error message will display in SureTyper: **There is no amplification which may be due to PCR inhibitors, a setup problem, or an unknown cause.**

LIMITATIONS OF THE PROCEDURE

- Optimal performance of the test requires correct sample set up. (See Specimen Collection and Preparation and Procedure for detailed information)
- All instruments must be calibrated according to manufacturer's instructions.
- The LinkSēq Wipe Test kits have been validated for use with the supplied reagents. Do not introduce other reagents except Molecular Biology Grade Water and sample as outlined in Specimen Collection and Preparation and Procedure.

QUALITY CONTROLS

A. Quality Controls

There are three types of quality control parameters within the LinkSēq Wipe Test Kits.

1. Inhibition Control Wells. There is an inhibition control well that must be run with each sample. The inhibition control has Control DNA added to the reaction buffer and allows the user to verify that no PCR inhibition is occurring. Possible inhibitors include ethanol, bleach, real-time PCR block issues etc.
2. Negative Control Wells. There are three negative control wells whose combined melt-curves determine the Negative Control end result. The Buffer A and Buffer B wells must detect no peak, and the Buffer Ag well must detect a peak in order for the Negative Control to pass. If any other combination occurs, the Negative Control will fail.
3. Positive Control Wells. There are three positive control wells whose combined melt-curves determine the Positive Control end result. All three wells must detect a peak in order for the Positive Control to pass. If any other combination occurs, the Positive Control will fail.

TRADEMARKS

LinkSēq Wipe Test kits are for Research Use Only—Not for use in diagnostic procedures. SYBR®, LinkSēq™ and SureTyper™ are trademarks of Thermo Fisher Scientific or its subsidiaries. LinkSēq products are covered by European Patent and Canada Patent 2603605. LinkSēq products may not be resold, distributed or repackaged.

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DISCLAIMERS

For Research Use Only. This product is not intended to provide information for the diagnosis, prevention or treatment of disease or to aid in the clinical decision making process. This product is not cleared or approved for clinical use by the FDA or approved in the EU as an in vitro diagnostic assay, nor is it CE marked.

EXPLANATION OF SYMBOLS

Symbol	Description
REF *ISO 7000 Reg No. 2493*	Catalog number
 *ISO 7000 Reg No. 1641*	Consult instructions for use* *Please consult Application Note for Research Use Only product
 *ISO 7000 Reg No. 0434A*	Caution, consult accompanying documents
 *ISO 7000 Reg No. 0632*	Temperature limitation
 *ISO 7000 Reg No. 3082*	Manufacturer
 *ISO 7000 Reg No 2497*	Date of Manufacture
 *ISO 7000 Reg No. 0518*	Contains sufficient for <n> tests
LOT	Batch Code

 *ISO 7000 Reg No 2607*	Use By Date

REVISION HISTORY

Revision	Date	Revision Description
01	Current	Transferred document contents to One Lambda's template. Updated the legal manufacturer and contact information.