LinkSēq™ HLA-B*57:01 Typing Kit Short Protocol

REF 8020C-SL, 8020C-SR

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

- 12 Strips containing dried reagents – BLUE Caps/ **BLUE Indicator Line**
- 2 vials of LS Buffer
- 1 vial of DNA Polymerase

Well ID	Sample Designation
A	Sample 1
B	Sample 1
C	Sample 2
D	Sample 2
E	Sample 3
F	Sample 3
G	Empty
H	NTC
—	Indicator Line

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 strip is completely sealed. Any openings in the strips can cause evaporation during amplification***
5. ***Use molecular biology grade water.***

Sample Setup Protocol

1. Remove the following reagents from freezer:
   a. LS buffer, DNA polymerase, and LinkSēq strip tubes.
   b. Once thawed keep LS Buffer and DNA polymerase chilled.
   c. Ensure contents are at the bottom of tubes prior to opening.
   d. After the strips have been equilibrated to room temperature, spin them down at 500-2,000 G for 30-60 seconds.

2. Track sample and strips identification on provided Strips Reference sheet for input into SureTyper™.
    a. If working with more than one type of test on strip tubes write the test name at the top tab of the strip after the storage caps (indicated by a color or color indicator line) caps have been removed.
    b. Write the column number in which the strips will be placed into the real time machine at the bottom tab of the strip after the storage caps have been removed.

    Note: the blue indicator line shows the position of the NTC well.
3. Prepare the sample in LS buffer vial:
    a. Add **12 µl** of DNA polymerase to the LS buffer vial.
    b. Close the vial lid and mix the reagents by inverting the tube 10 times or pulse-vortexing for 5 seconds.

    Note: The amount of reagent is enough for 6 strips. Store the LS buffer vial with DNA polymerase at 2-8°C. Use reagents within 3 months.
4. Remove the storage caps and label strips appropriately.
5. Add 5 µl of the LS buffer (containing DNA polymerase) to each well in use.
6. Add 5 µl of molecular biology grade water to the No Template Control (NTC) in well H.
7. Add 5 µl of DNA and molecular biology grade water to each test well in use that is not the NTC well. (Total DNA between 8 ng to 100 ng to each test well)
    a. If DNA concentration is between 1.6 ng/µl and 20 ng/µl, add 5µl of DNA solution to each well.
    b. If DNA concentration is between 4 ng/µl and 50 ng/µl, add 2 µl of sample and 3 µl of molecular biology grade water to each well.
    c. If DNA concentration is less than 1.6ng/µl, then increase DNA concentration before performing test.
8. Seal the strips with PCR Optical Strip Caps provided.
9. Spin the strip wells down so that all the reagents are at the bottom of the wells and begin Step 10 immediately.
10. Set the strips(s) in the thermal cycler or real-time PCR instrument. If using a thermal cycler, follow the instructions supplied by the manufacturer. Begin amplification.
    Caution: When placing the strips into the real-time PCR instrument, be sure to place them in the column location(s) noted on the plate reference sheet in step 2 above. The column information will be important for later data analysis.
11. If amplification is performed on a thermal cycler, remove the tray any time during the 4°C hold step and dissociate on the real-time PCR instrument.
12. After the amplification and dissociation are complete, export the data from the real-time PCR instrument according to the instructions in the SureTyper™ Manual.

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