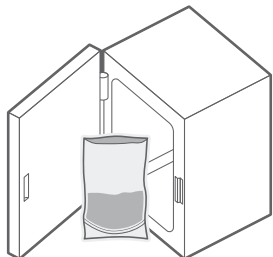


Ready Reference for Real-Time PCR Assays*

keydiagnostics
 T: 02 8212 4074 F: 02 9423 6992
 info@keydiagnostics.com.au
 www.keydiagnostics.com.au
 PO Box 1038, Gympie, NSW, 2227

* For Real-Time *Listeria* assays, see other side

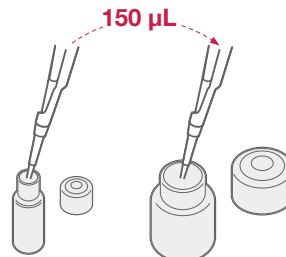
Enrich samples.
(See User Guide)



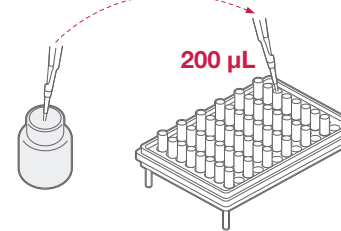
1. Create rack file with data on each sample.



2. Add 150 µL protease to 12 mL lysis buffer.

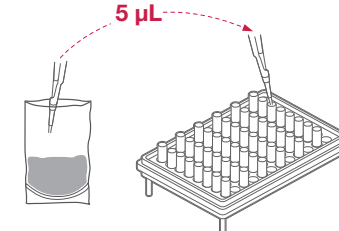


3. Add 200 µL lysis reagent from step 2 to cluster tubes.



Lysis reagent can be stored at 2-8 °C for up to two weeks

4. Transfer 5 µL* enriched samples to cluster tubes.



For *E. coli* O157:H7 and STEC, use 20 µL

5a. Heat cluster tubes (First Stage*)

0:20 **37°C for 20 minutes:**
Campylobacter
E. coli O157:H7
E. coli - STEC suite
Salmonella
Shigella
Vibrio

1:00 **55°C for 60 minutes:**
Staphylococcus aureus

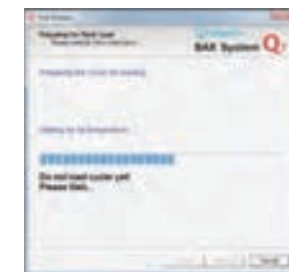
5b. Heat cluster tubes (Second Stage)

0:10 **95 °C for 10 minutes:**
 All targets

6. Cool cluster tubes 5 minutes in cooling block.

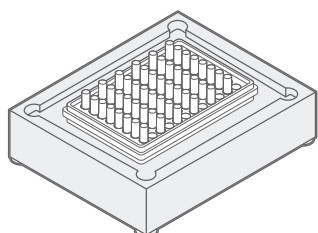
0:05

7. Initialize cycler/detector.

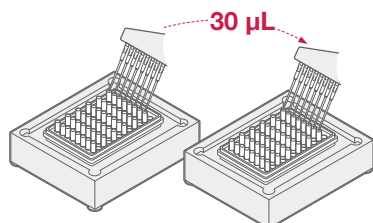


* Steps 5 and 6 can also be performed using the **Hygiena[™] Automated Thermal Block**. See the Automated Thermal Block User Guide for details and instructions.

8. Arrange PCR tubes in PCR cooling block.



9. Hydrate PCR tablets with 30 µL lysate from step 6.*



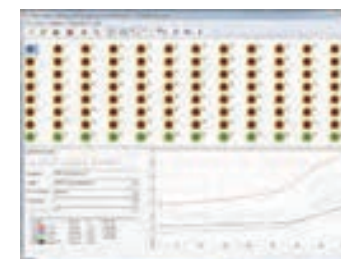
10. Place PCR tubes in cycler and run program.



For Real-Time *Salmonella* and *E. coli* O157:H7 Exact, let the hydrated PCR tubes sit in the cooling block for 10-30 minutes before loading into BAX[®] Q7 instrument.

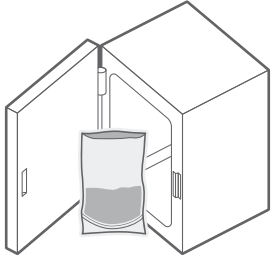
11. Review results on screen. See User Guide for details.

- Negative
- Positive
- Indeterminate
- Signal error

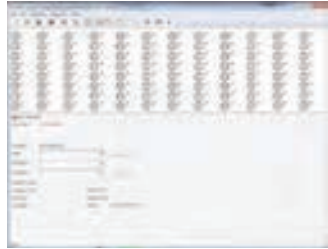


Ready Reference for Real-Time *Listeria* PCR Assays

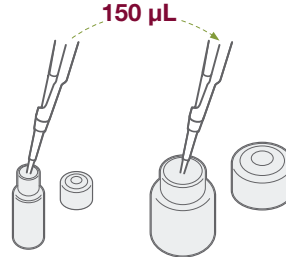
Enrich samples.
(See User Guide)



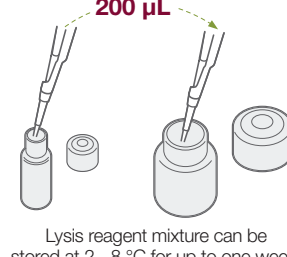
1. Create rack file with data on each sample.



2. Add 150 µL protease to 12 mL lysis buffer.

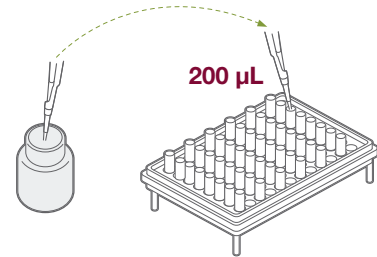


3. Add 200 µL Lysing Agent 2 to protease and lysis buffer mixture.

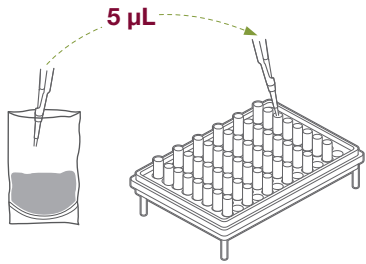


Lysis reagent mixture can be stored at 2 - 8 °C for up to one week.

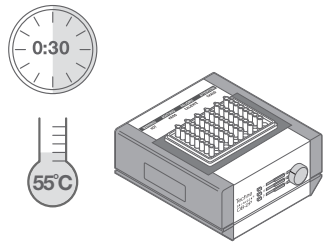
4. Add 200 µL lysis reagent from step 3 to cluster tubes.



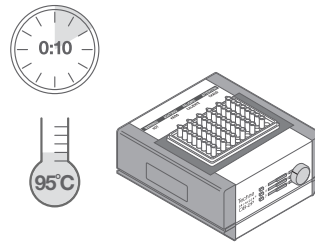
5. Transfer 5 µL enriched sample to cluster tubes.



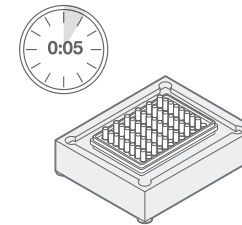
6. Heat cluster tubes at 55 °C for 30 minutes.



7. Heat cluster tubes at 95 °C for 10 minutes.



8. Cool cluster tubes in chilled cooling block for 5 minutes.

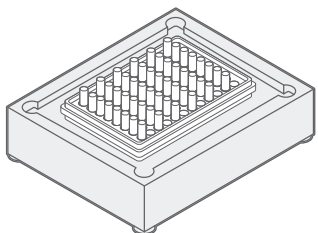


9. Initialize cycler/detector.

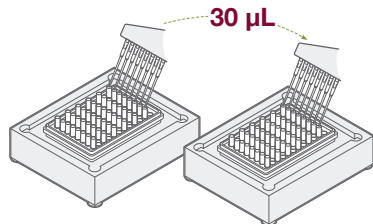


* Steps 6 - 8 can also be performed using the Hygiena™ Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.

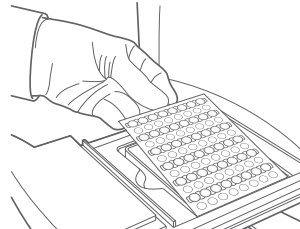
10. Arrange PCR tubes in cooling block.



11. Hydrate PCR tablets with 30 µL lysate from step 8.*

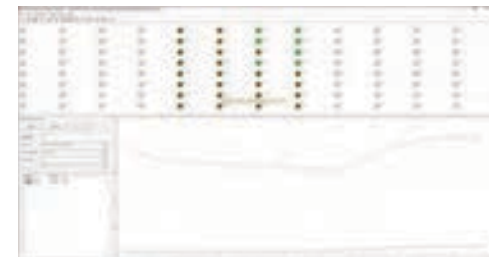


12. Place tubes in cycler/detector and run program.



11. Review results on screen. See User Guide for details.

- Negative
- Positive
- Indeterminate
- Signal error



* An optional 10-30 minute hold of the hydrated PCR tablets in the cooling block is recommended for the Real-Time *Listeria* assays in order to be consistent with the Real-Time *Salmonella* procedure.