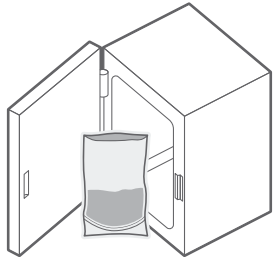


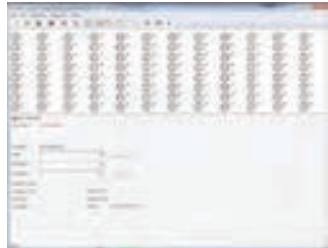
# Ready Reference for Standard PCR Assays

**keydiagnostics**  
 T: 02 8212 4074 F: 02 9423 0992  
 info@keydiagnostics.com.au  
 www.keydiagnostics.com.au  
 PO Box 2038, Gymer, NSW, 2227

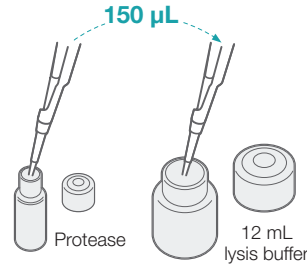
Enrich samples.  
(See User Guide)



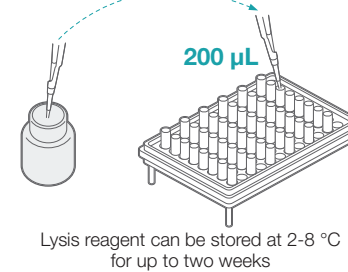
1. Create rack file with data on each sample.



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

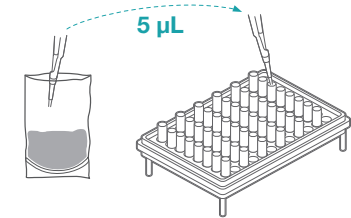


3. Add 200 µL lysis reagent from step 2 to cluster tubes.



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

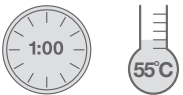
\*20 µL for *E. coli* O157:H7 samples enriched with BAX System MP Media



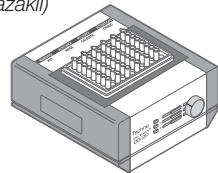
5a. Heat cluster tubes (First Stage\*)



**37°C for 20 minutes for Gram negative organisms**  
*E. coli* O157:H7 • *E. coli* - STEC suite  
*Salmonella* • *Cronobacter* (*E. sakazakii*)

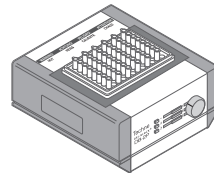


**55°C for 60 minutes for Gram positive organisms**  
 Genus *Listeria*  
*L. monocytogenes*

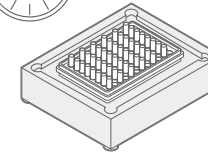


5b. Heat cluster tubes (Second Stage)

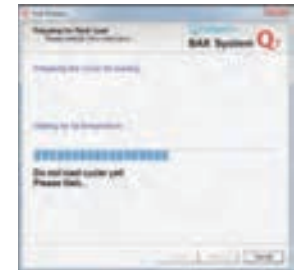
95 °C for 10 minutes:



6. Cool cluster tubes 5 minutes in cooling block.

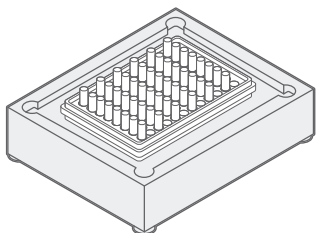


7. Initialize cycler/detector.

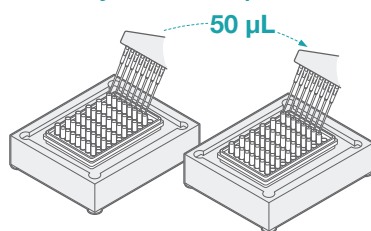


\* Steps 5 and 6 can also be performed using the **Hygiena™ Automated Thermal Block**. See the Automated Thermal Block User Guide for details and instructions.

8. Arrange PCR tubes in PCR cooling block.



9. Hydrate PCR tablets with 50 µL lysate from step 7.\*



10. Place PCR tubes in cycler and run program.



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

- Negative
- Positive
- Indeterminate
- Signal error

