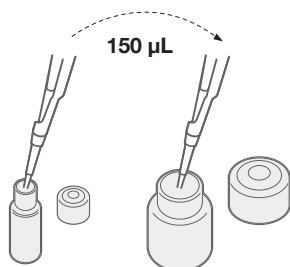


Ready Reference for Standard PCR Assays

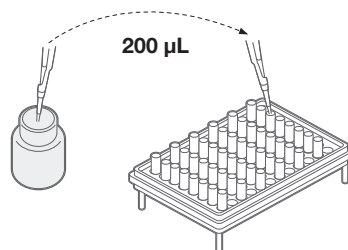
keydiagnostics
 T: 02 8212 4974 F: 02 9423 6992
 info@keydiagnostics.com.au
 www.keydiagnostics.com.au
 PO Box 1038, Gympie, NSW, 2227

STEP 1: PREPARATION

Add 150 µL protease to 12 mL lysis buffer

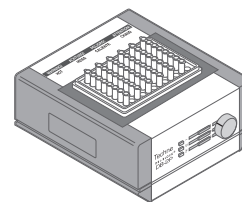


Add 200 µL lysis reagent to cluster tubes

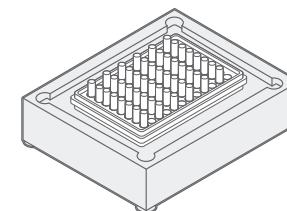


Lysis reagent can be stored at 2-8°C for up to two weeks

Ensure thermal blocks are pre-heated to 37°C or 55°C, and 95°C prior to use

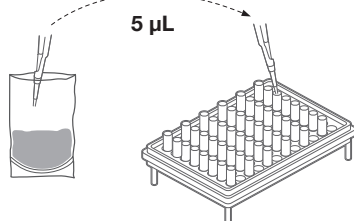


Ensure cooling blocks are stored at 2 – 8°C prior to use



STEP 2: LYSIS

Transfer 5 µL* enriched samples to cluster tubes

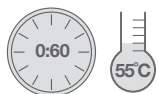


*For *E. coli* O157:H7 use 20 µL

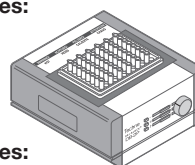
Heat cluster tubes (First Stage)



37°C for 20 minutes:
Cronobacter
E. coli O157:H7
Salmonella



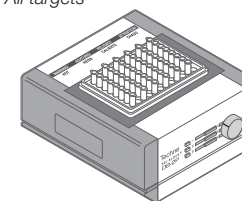
55°C for 60 minutes:
 Genus *Listeria*
L. monocytogenes



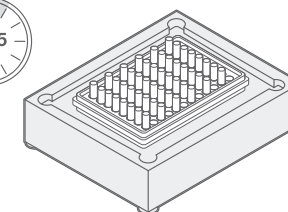
Heat cluster tubes (Second Stage)



95°C for 10 minutes:
 All targets



Cool cluster tubes for a minimum of 5 minutes in cooling block



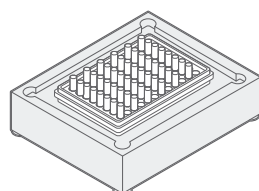
Unopened processed lysates can be stored at 2-8°C for up to two weeks

STEP 3: PCR

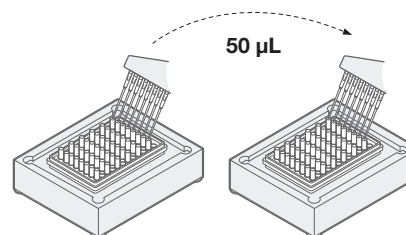
Create rack file, turn on cycler, and initialize



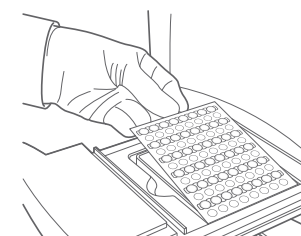
Arrange PCR tubes in PCR cooling block with black carrying tray



Hydrate PCR tablets with 50 µL lysate from cooled lysates



On software, click next, place PCR tubes in Q7 cycler and run program



Review results on screen

-  Negative
-  Positive
-  Indeterminate
-  Signal error