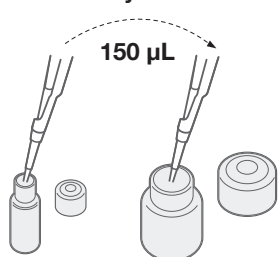


Ready Reference for Real-Time PCR Assays*

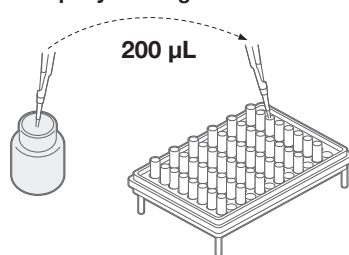
keydiagnostics
 T: 02 8422 4074 F: 02 9423 6992
 info@keydiagnostics.com.au
 www.keydiagnostics.com.au
 PO Box 1038, Gymea, NSW, 2227

STEP 1: PREPARATION

Add 150 µL protease to
12 mL lysis buffer

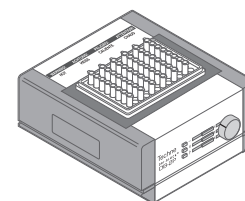


Add 200 µL lysis reagent to cluster tubes

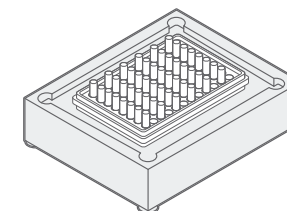


Lysis reagent can be stored at
2-8°C for up to two weeks

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

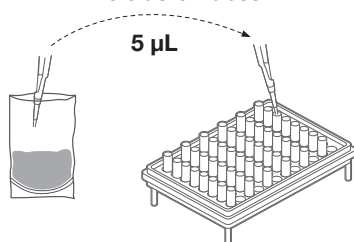


Ensure cooling blocks are
stored at 2 – 8°C prior to use



STEP 2: LYSIS

Transfer 5 µL* enriched samples
to cluster tubes

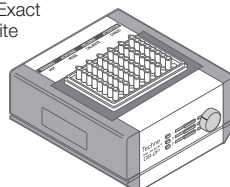


*For *E. coli* O157:H7 and STEC, use 20 µL

Heat cluster tubes (First Stage)



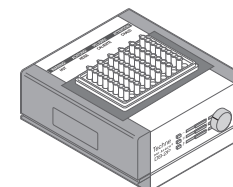
37°C for 20 minutes:
Campylobacter
E. coli O157:H7 Exact
E. coli - STEC suite
Salmonella
Shigella
Vibrio



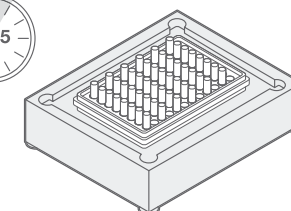
Heat cluster tubes (Second Stage)



95°C for 10 minutes:
 All targets



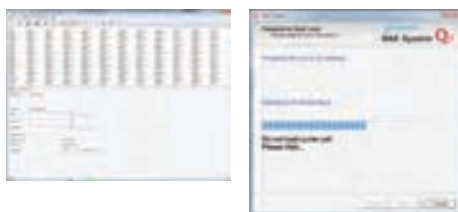
Cool cluster tubes for a minimum of
5 minutes in cooling block



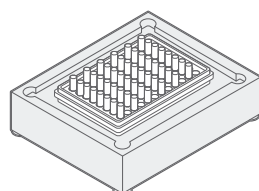
Unopened processed lysates can
be stored at 2-8°C for up to two weeks

STEP 3: PCR

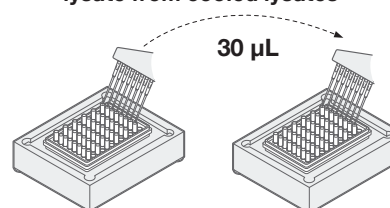
Create rack file, turn on cycler,
and initialize



Arrange PCR tubes in
PCR cooling block with
black carrying tray

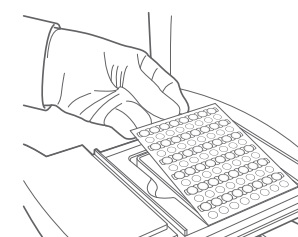


Hydrate PCR tablets with 30 µL
lysate from cooled lysates



For Real-Time *Salmonella* and *E. coli* O157:H7 Exact,
let hydrated tablets sit in the cooling block for
10-30 minutes prior to placing tubes in Q7 Cycler.

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



Review results on screen

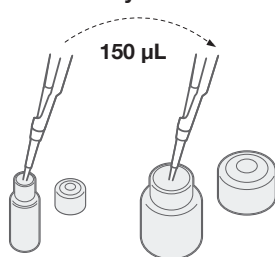


Ready Reference for Real-Time *Listeria* PCR Assays

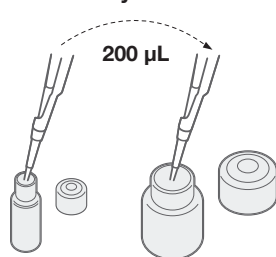
keydiagnostics
 T: 02 8212 4074 F: 02 9423 6992
 info@keydiagnostics.com.au
 www.keydiagnostics.com.au
 PO Box 1038, Gymea, NSW, 2227

STEP 1: PREPARATION

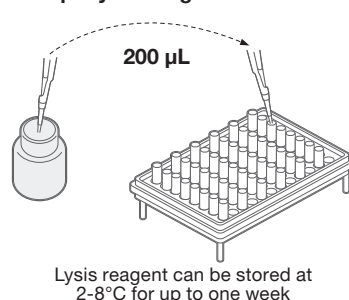
Add 150 µL protease to
12 mL lysis buffer



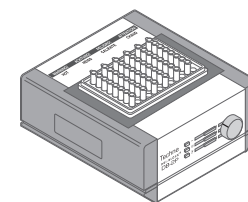
Add 200 µL Lysing Agent 2 to
protease and lysis buffer mixture



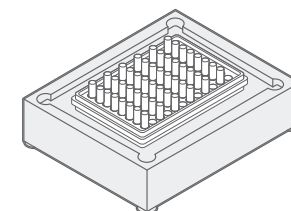
Add 200 µL lysis reagent to cluster tubes



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

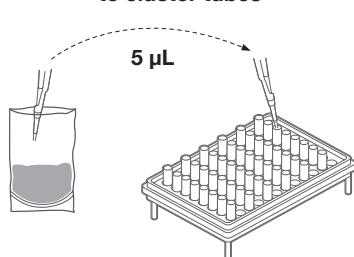


Ensure cooling blocks are
stored at 2 – 8°C prior to use



STEP 2: LYSIS

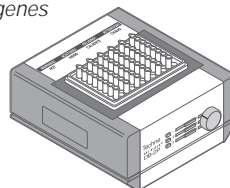
Transfer 5 µL enriched samples
to cluster tubes



Heat cluster tubes (First Stage)



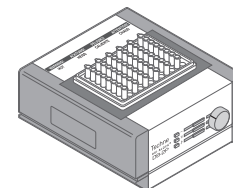
55°C for 30 minutes:
Genus *Listeria*
L. monocytogenes



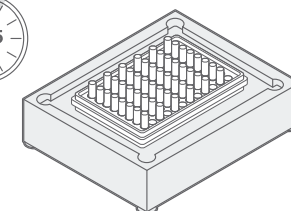
Heat cluster tubes (Second Stage)



95°C for 10 minutes:
All targets



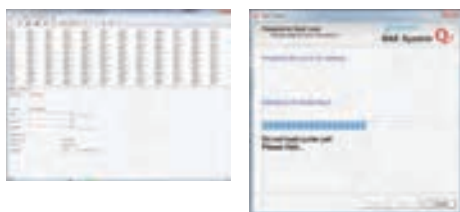
Cool cluster tubes for a minimum of
5 minutes in cooling block



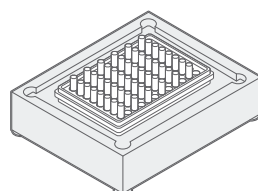
Unopened processed lysates can
be stored at 2-8°C for up to two weeks

STEP 3: PCR

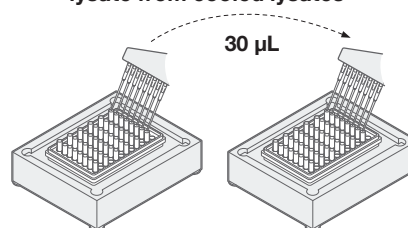
Create rack file, turn on cycler,
and initialize



Arrange PCR tubes in
PCR cooling block with
black carrying tray

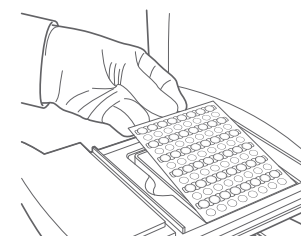


Hydrate PCR tablets with 30 µL
lysate from cooled lysates






Recommended: 10 - 30 min hold in cold block for
hydrated tablets prior to placing in Q7 cycler

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



Review results on screen

-  Negative
-  Positive
-  Indeterminate
-  Signal error