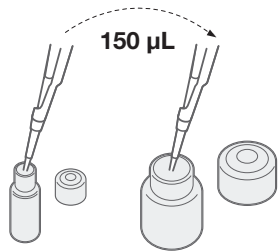


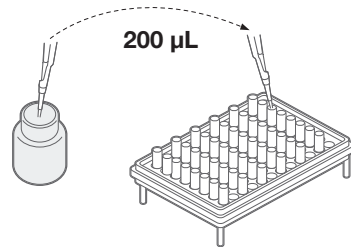
Ready Reference for Real-Time PCR Assays*

STEP 1: PREPARATION

Add 150 µL protease to 12 mL lysis buffer

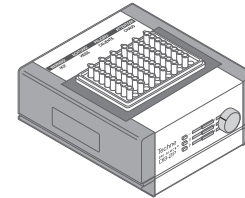


Add 200 µL lysis reagent to cluster tubes

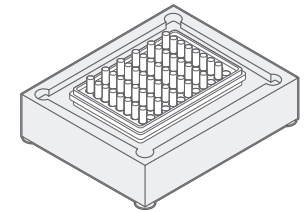


Lysis reagent can be stored at 2-8°C for up to two weeks

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

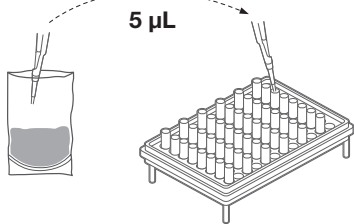


Ensure cooling blocks are stored at 2 – 8°C prior to use



STEP 2: LYSIS

Transfer 5 µL* enriched samples to cluster tubes



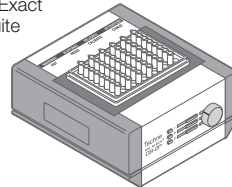
*For *E. coli* O157:H7 and STEC, use 20 µL

Heat cluster tubes (First Stage)



37°C for 20 minutes:

Campylobacter
E. coli O157:H7 Exact
E. coli - STEC suite
Salmonella
Shigella
Vibrio

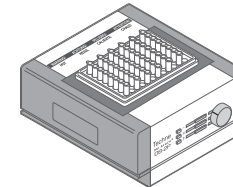


Heat cluster tubes (Second Stage)

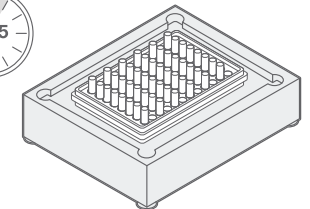


95°C for 10 minutes:

All targets



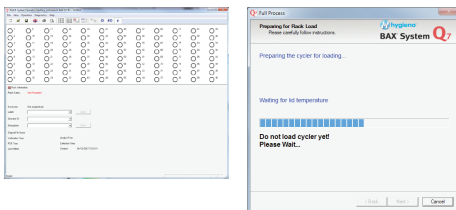
Cool cluster tubes for a minimum of 5 minutes in cooling block



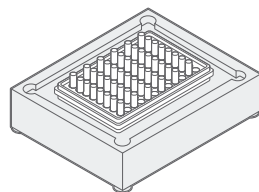
Unopened processed lysates can be stored at 2-8°C for up to two weeks

STEP 3: PCR

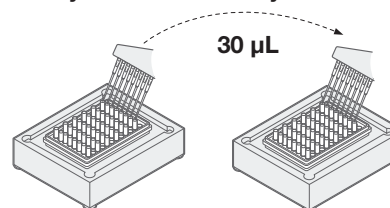
Create rack file, turn on cycler, and initialize



Arrange PCR tubes in PCR cooling block with black carrying tray

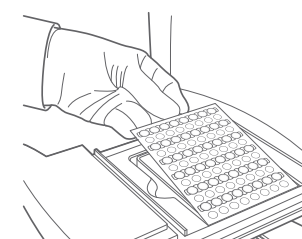


Hydrate PCR tablets with 30 µL lysate from cooled lysates



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



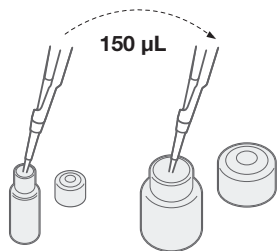
Review results on screen

- Negative
- Positive
- Indeterminate
- Signal error

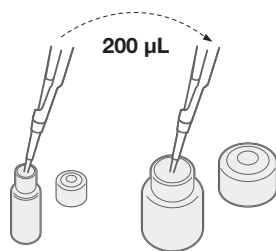
Ready Reference for Real-Time *Listeria* PCR Assays

STEP 1: PREPARATION

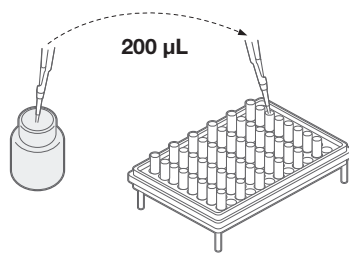
Add 150 µL protease to 12 mL lysis buffer



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

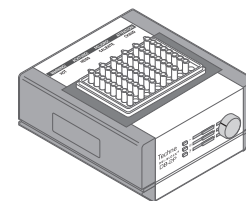


Add 200 µL lysis reagent to cluster tubes

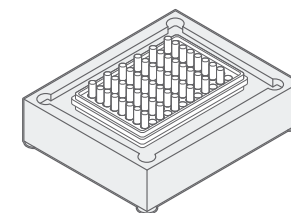


Lysis reagent can be stored at 2-8°C for up to one week

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

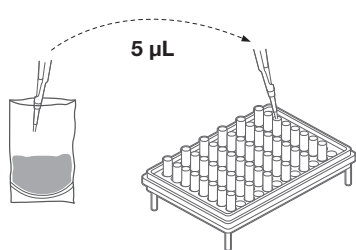


Ensure cooling blocks are stored at 2 – 8°C prior to use



STEP 2: LYSIS

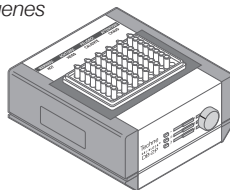
Transfer 5 µL enriched samples to cluster tubes



Heat cluster tubes (First Stage)



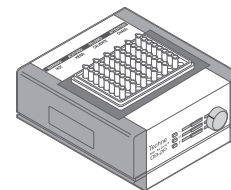
55°C for 30 minutes:
Genus *Listeria*
L. monocytogenes



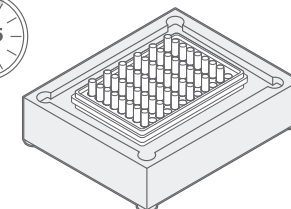
Heat cluster tubes (Second Stage)



95°C for 10 minutes:
All targets



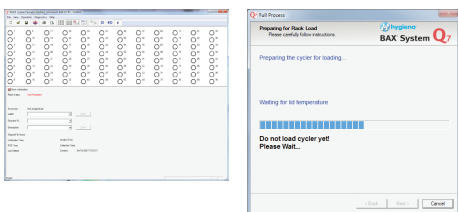
Cool cluster tubes for a minimum of 5 minutes in cooling block



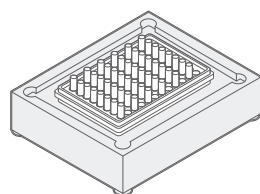
Unopened processed lysates can be stored at 2-8°C for up to two weeks

STEP 3: PCR

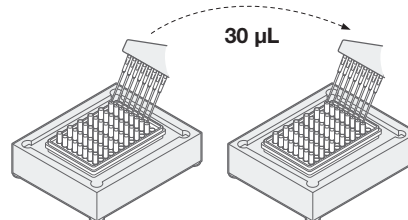
Create rack file, turn on cycler, and initialize



Arrange PCR tubes in PCR cooling block with black carrying tray

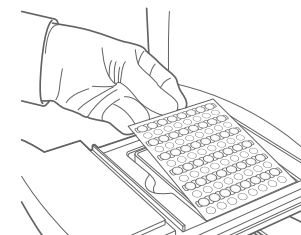


Hydrate PCR tablets with 30 µL lysate from cooled lysates



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