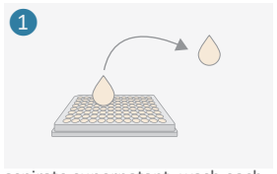
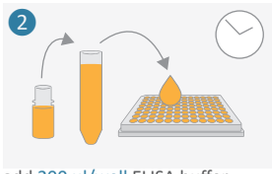
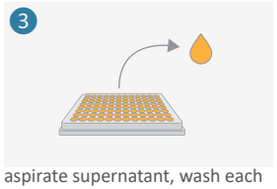


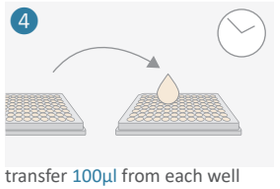
aspirate supernatant, wash each well once with 200µl wash buffer



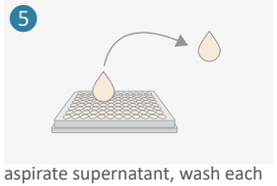
add 200 µl/well ELISA buffer, incubate for 1h



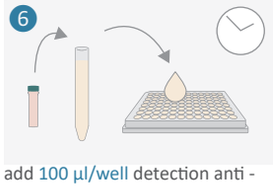
aspirate supernatant, wash each well once with 200µl wash buffer



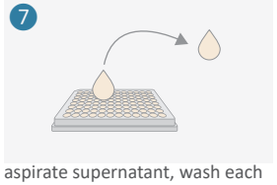
transfer 100µl from each well of the assay plate to the ELISA plate, incubate for 2h



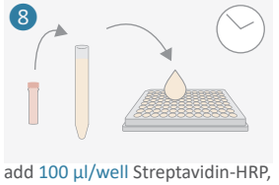
aspirate supernatant, wash each well once with 200µl wash buffer



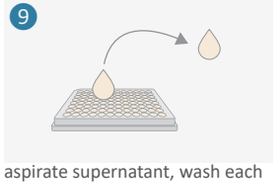
add 100 µl/well detection anti - body, incubate for 1h



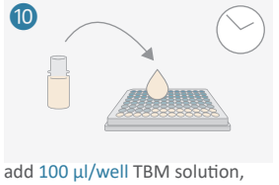
aspirate supernatant, wash each well once with 200µl wash buffer



add 100 µl/well Streptavidin-HRP, incubate for 30min

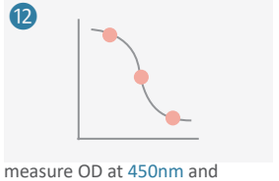


aspirate supernatant, wash each well thrice with 200µl wash



add 100 µl/well TBM solution, incubate for 15min in the dark

add 100 µl/well stop solution



measure OD at 450nm and 570nm for reference

day III: ELISA Assay

Perform each incubation step on a plate shaker.

- Prepare 150ml of wash buffer (0.05% Tween20 in PBS).
- Dilute ELISA Buffer 1:5 to 50ml in ddH_2O.

- Aspirate wells of the ELISA plate and wash once with 200 µl/well of wash buffer. 1
- Add 200µl ELISA buffer to each well of the ELISA plate. Incubate for 1h at room temperature. 2
- Aspirate wells and wash once with 200 µl/well of wash buffer. 3
- Transfer 100µl from the Assay plate to corresponding wells of the ELISA plate. Incubate for 2h at room temperature. 4
- Dilute the detection antibody 1:250 in ELISA buffer (42µl + 10.458ml).
- Aspirate the supernatant of the ELISA plate and wash once with 200µl washing buffer. Let the buffer soak in for 1min. 5
- Add 100µl detection antibody to each well and incubate for 1h at room temperature. 6
- Dilute Streptavidin-HRP 1:100 in ELISA buffer (110µl + 10.890ml).
- Aspirate the supernatant and wash once with 200µl washing buffer. Let the buffer soak in for 1min. 7
- Add 100µl Streptavidin-HRP to each well and incubate for 30min at room temperature. 8
- Aspirate the supernatant and wash thrice with 200µl washing buffer. Let the buffer soak in for 1min during every step. 9
- Add 100µl TMB solution to each well and incubate for 15min at room temperature while shaking in the dark. 10
- Add 100µl of stop solution (1M H_2SO_4) to each well. 11
- Measure OD at 450nm and 570nm for reference. 12

For analysis and acceptance criteria, please refer to the product information of the instaCELL pyrogen detection kit.

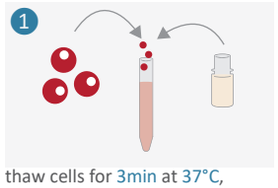
acCELLerate®

instaCELL® pyrogen detection kit (MAT)

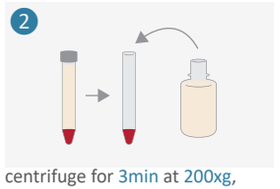
protocol

SAFETY

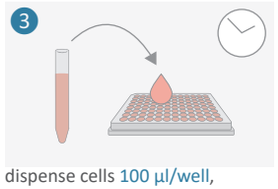
Version 1.0



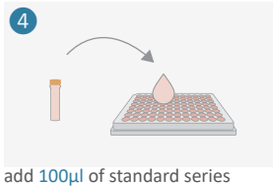
thaw cells for 3min at 37°C, dilute in 8ml recovery buffer



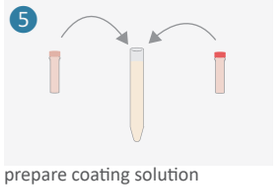
centrifuge for 3min at 200xg, resuspend in 10ml assay buffer



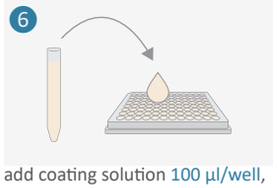
dispense cells 100 µl/well, incubate for 24h



add 100µl of standard series and sample to each corresponding well, incubate for 18h



prepare coating solution



add coating solution 100 µl/well, incubate overnight at 4°C

The instaCELL pyrogen detection kit can be conducted in three different versions:
A. Quantitative Test
B. Semi-Quantitative Test
C. Reference Lot Comparison Test
This protocol refers to the Quantitative Test. For information on methods B, C and the product-specific validation to determine the maximum valid dilution (MVD), please refer to the product information on: www.accellerate.me/support/downloads

day I: preparation of cells

- Keep the cells on dry ice before thawing and process quickly.
- Add 60µl of 50mM beta-mercaptoethanol to the assay buffer.
- Equilibrate the assay and recorvery buffer to 37°C.
- Thaw one vial of Assay Ready Cells in a water bath at 37°C for 3min. Prepare 8ml of recovery buffer in a 15ml centrifugation tube. Dispense the cells completely into the prepared tube. Rinse the cryovial once with 1ml recovery buffer. 1
- Centrifuge for 3min at 200xg and carefully aspirate the supernatant. Resuspend the cell pellet in 10ml of assay buffer. 2
- Dispense 100µl of the cell suspension into each well of the provided assay plate. 3
- Incubate for 24h in a humidified incubator at 37°C and 5% CO_2.

day II: preparation of test chemicals and ELISA plate

- Dilute the BRP stock solution 1:25 in assay buffer to obtain the BRP working solution. Dilute the working solution 1:50 to obtain C1 of the BRP standard curve and perform 2-fold serial dilutions for a total of 7 dilutions (see plate layout).
- Each sample is tested in three concentrations. Use the concentration previously determined in the test for interfering factors as the highest sample concentration and prepare two-fold serial dilutions for concentrations 2 and 3, not exceeding the MVD.
- Prepare your sample in assay buffer at 2x of the final concentration.
- Dilute the BRP working solution 1:4 to obtain the spiking solution. Add 10µl of spiking solution to 990µl of the test sample dilutions to spike your samples.
- Add 100µl of BRP standard series, samples, spiked samples and blank in quadruplicates to the respective wells of the assay plate. Use Assay Buffer for the blank. Incubate for 18h in a humidified incubator at 37°C and 5% CO_2. 4

- Dilute coating buffer 1:10 in cell culture water (1.1ml + 9.9ml). Dilute capture antibody 1:250 in coating buffer (42µl + 10458µl). 5
- Coat the provided 96-well ELISA plate by adding 100µl of coating solution to each well. Incubate overnight at 4°C. 6

kit content

- Assay Ready Cells (1x vial)
- Assay Buffer A
- Recovery Buffer A
- Coating Buffer
- Strepdavidin-HRP
- ELISA Buffer
- TMB Solution
- 96-well plates
- Detection Antibody
- Capture Antibody
- BRP-Standard

not provided

DPBS
Tween 20
1M H_2SO_4
50mM ß-mercaptoethanol

storage

Store Assay Ready Cells in liquid nitrogen (below -140°C)
Store all reagents and media at temperatures indicated on the label

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