Stanford Human Immune Monitoring Core
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Luminex Multiplex Analysis

1. Principle

Measurment of cytokines, chemokines and growth factors is achieved in a fluid based sample of human or other origin. Multiple targets are measured and analyzed simultaneously from a single small volume of sample and detection is at the pg/ml for most targets and has a 3-4 log dynamic range.

2. Materials and Equipment

Please refer to attached Assay Manual

3. Procedure

A. Sample preparation

For sample preparation please refer to Manual attached according to sample type**.**

B. Luminex assay

Preparation of Standards and Samples for assay

Thaw samples at room temperature and ensure they reach RT (Room Temperature) before adding to 96 well filter plate for assay. Do not leave samples at RT for long periods prior to loading as sample maybe compromised. Place on ice if longer than 30 minutes at RT**.**

I- Standard preparation- Follow manual to prepare. We prepare a large batch of standards by rehydrating 4-8 lyophilized standard tubes with 250ul of buffer.

a. Vortex each vial for 10 seconds.
b. Incubate on ice for 25minutes.
c. Combine all vials of standards and incubate for additional 5'on ice.
d. Apportion 70ul aliquots and freeze in -80 promptly for later use.
e. Thaw Standard aliquot ~10 minutes before use. Incubate on Ice for 5 minutes after thaw and before serial dilution.

Follow assay protocol details below:

Step 1. Pre-Wet Filter Plate
a. Add 120 ul/well of Reading Buffer
b. Incubate for 5 -10 min
c. Remove the buffer by filtration

IMPORTANT Following the last wash in each series, blot the bottom of the Filter Plate thoroughly with a paper towel.
IMPORTANT Place the Filter Plate on the bench during all processing and place in aluminum foil wrapped around during incubation and shaking processing steps. Room temperature incubation steps are performed on an orbital shaker at 500-600 rpm.

Step 2. Add Prepared Samples and Antigen Standards
a. Add 25 uL/well of Assay Buffer
b. Add 25 uL/well of samples or Antigen Standards

Step 3. Add Antibody Beads
a. In a 15 ml tube Add 5.5ml of assay buffer. Add Antibody Beads (~120ul) and Assaychex beads (50ul) into the 5.5ml assay buffer and vortex for 30 sec. Place in reservoir and apply 50 uL/well (multichannel).
b. Seal plate and incubate at 20-25 ºC for 2 hours shaking at 500 rpm
c. Transfer plate to 4ºC refrigerator for overnight incubation.
d. Next day-Pre-warm plate for 20-30 minutes at room temperature without shaking
e. Remove solution by filtration
f. Wash 3 times using 150 uL/well of 1X Wash Buffer

Step 4. Add Detection Antibody
a. Add 25 uL/well of Detection Antibody to each well
b. Seal plate and incubate at 20-25 ºC for 2 hours shaking at 500 rpm
c. Remove solution by filtration
d. Wash 3 times using 150 uL/well of 1X Wash Buffer

Step 5. Add Streptavidin-PE
a. Add 50 uL/well of Streptavidin-PE

b. Seal plate and incubate at 20-25 ºC for 40 min shaking at 500 rpm
c. Remove solution by filtration
d. Wash 3 times using 150 uL/well of 1X Wash Buffer

Step 6. Read Plate
a. Add 120 uL/well of Reading Buffer
b. Seal plate and incubate at 20-25 ºC for 5 min shaking at 500 rpm
c. Remove seal and read on Luminex instruments according to specification of kit.

Luminex startup and shutdown menues should be followed. Calibration and Control testing is performed once on the day of Luminex run prior to sample run (unless there are flow or other clogging problems).

Report and Data analysis

Output CSV file is used to extract data from BeadView, Bioplex or Masterplex QT4 software.

We prepare a folder per customer containing : A report file, MFI and Concentration graphs as well as 4-6 standard graphs according to targets detected in samples.

Report file contains all information of standards, Curve fitting, Bead counts to examine quality of assay and CV of both samples and standards.

In some large data sets standard comparison across plates is performed as well as analysis using TM4/Mev (free software)