Protocol 508_Human/mouse Soluble Protein Master Buffer Kit Protocol

Materials:
- BD CBA Soluble Protein Flex Set
- Soluble protein master buffer kit
- Non-sterile polystyrene V Bottom plates (BD Falcon 353263)

Preparation of Soluble Protein Flex Set Standards

Resuspend the desired lyophilized standard spheres in 4.0 ml Assay Diluent to obtain a final concentration of 2500 pg/ml. Mix reconstituted protein by pipette only (**do not vortex**)
Perform 1:2 serial dilutions by transferring 75 μl of Top Standard into 75 μl Assay diluent and so on
With remaining volume of Top standard, make 150 μl aliquots and freeze at 80°C

	Top Standard	1:2 dilution tube	1:4 dilution tube	1:8 dilution tube	1:16 dilution tube	1:32 dilution tube	1:64 dilution tube	1:128 dilution tube	1:256 dilution tube
Protein (pg/ml)	2500	1250	625	312.5	156	80	40	20	10

Preparation of Test Samples

Transfer 25 μl of the diluted serum or culture supernatant to the V-Bottom plate.

Serum samples: diluted 1:4 in Assay Diluent (7.5 μl serum in 22.5 μl Assay diluent – can be done directly on the assay plate).
Supernatant from cell culture can be used neat. It is recommended to freeze supernatants as aliquots because freeze-thaw cycles can degrade proteins.

Preparation of BD CBA Protein Flex Set Capture Beads

Capture Beads are at a 50× concentration and must be diluted to their optimal concentration before adding to an assay well.
- Vortex each Capture Bead stock vial for at least 15 seconds to resuspend beads thoroughly
- Determine the total volume of diluted beads needed
  Each well requires 25 μl of diluted beads:
  Eg: 35 tests × 25 μl = 875 μl
- Determine the volume needed for each Capture Bead
  Each test requires 0.5 μl.
  Eg: 35 tests × 0.5 μl = 17.5 μl
- Prepare the cocktail:
  If testing 1 analyte: 875 μl – (17.5 μl × 1) = 857.5 μl
  If testing 5 analytes: 875 μl – (17.5 μl × 5) = 787.5 μl

Preparation of BD CBA Soluble protein Flex Set PE Detection Reagents

PE Detection Reagents are at a 50× concentration and must be diluted to their optimal concentration before adding to an assay well.

- Determine the total volume of diluted PE Detection Reagents needed
  Each well requires 25 μl of diluted PE Detection Reagents:
  Eg: 35 tests × 25 μl = 875 μl
- Determine the volume needed for each PE Detection Reagents
  Each test requires 0.5 μl.
  Eg: 35 tests × 0.5 μl = 17.5 μl
- Prepare the cocktail:
  If testing 1 analyte: 875 μl – (17.5 μl × 1) = 857.5 μl
  If testing 5 analytes: 875 μl – (17.5 μl × 5) = 787.5 μl

Assay

- Add 25 μl diluted capture beads mix to each test (standard + samples)
- Incubate for 1 hour at 4°C, protected from light
- Wash the beads with 200μl wash buffer
- Centrifuge at 1500 rpm for 5 min, flick off the supernatant
- Add 25 μl PE Detection Reagent mix to each test (standard + samples)
- Incubate for 1 hour at 4°C, protected from light
- Wash the beads with 200 μl wash buffer
- Centrifuge at 1500 rpm for 5 min, flick off the supernatant
- Resuspend in 200 μl wash buffer

FACS Array Acquisition and Analysis

Acquisition:
Open FACS Array software
Login: Emma Slack (no password)

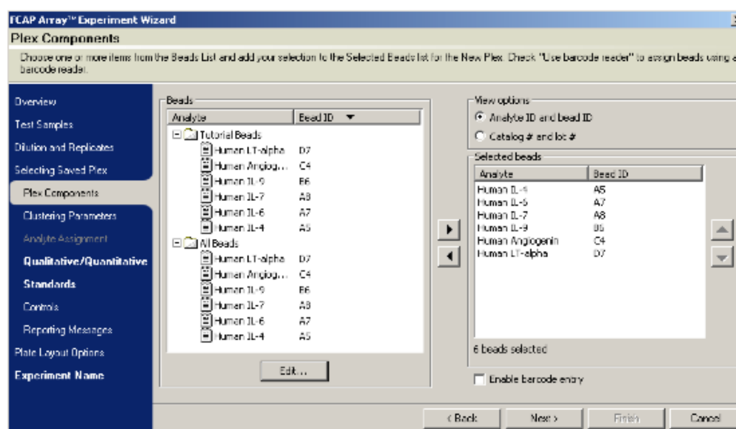
- Click Experiment Wizard (on the top left-hand side)
- *Setting*: Default
- *Template*: CBA Maria
- Add the number of samples
- All other specifications do not need to be altered from the standard settings
- Load the plate and acquire
- Export FCS files to the C:Drive

FCAP Array CBA Analysis

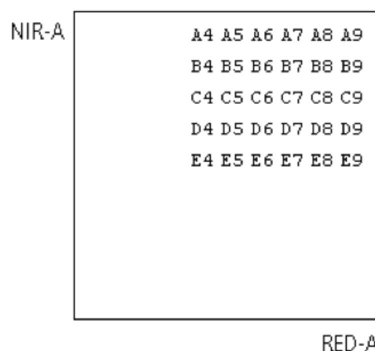
Designing an Experiment

Open FCAP Array, password: welcome

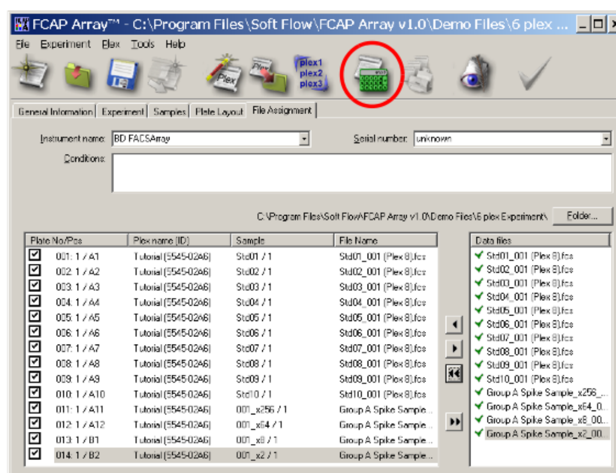
- Click the *New experiment* icon – the opening view of the Experiment Wizard is displayed
- Add the number of Test Samples used in the CBA
- Specify a dilution factor for each of the test samples
- Chose the analytes used in your plex



- Import a FCS file and specify the machine used for the acquisition (FACS Array) to detect the bead clusters
- Verify that clustering has succeeded and that the number of clusters is equal to the number of analytes added in the previous step
- Assign analytes to displayed



- Click next and choose *quantitative* and *4-parameter logistic*
- Click next and enter the concentration for each standard
- Click the *File Assignment* tab. Assign the corresponding FCS files to the Plex ID on the left
- Click the *Start analyzing this experiment* icon



Notes:

If the FCAP Array software is unable to recognize all the bead clusters because of debris, use the **FCS filter software**. Filter the bead clusters without debris according to the software instructions and save them.

Important! Export FCS2.0 FCS files (otherwise you won't be able to view the plots in a logarithmic scale).