Cytokine Array: Host and Tumour Profiling unit, Cancer Research UK - Edinburgh Centre, IGMM.

If you are considering using the service:

The cytokine assay is set up to determine relative expression of 64 targets selected from our in-house lists of human or mouse antibody stocks.

1) Standard array layout: 64 capture antibodies printed as 64 sub-arrays with 4 replicates per target on 1 array slide. The assay is performed with 1 slide per sample.

2) The assay is performed with a minimum of 10 samples and up to 20 samples can be processed per day. One control sample per experiment must be included. (For more than 20 samples please discuss further with HTPU).

3) For pricing and scheduling of studies contact the Host and Tumour Profiling Unit: cytokine@igmm.ed.ac.uk

4) For further technical advice on the assay please contact the FPA Technical Manager: Kenneth.Macleod@igmm.ed.ac.uk.

Running Cytokine Arrays at HTPU, IGMM:

1) Once you have decided to proceed with a study, contact HTPU for pricing and scheduling of your study. For clients located outwith The University of Edinburgh a Supply of Services Agreement must be signed by both parties.

2) Ideally, 3ml volume per sample should be provided on dry ice to HTPU. We can proceed with lower volumes by adjusting to 3ml with PBS, but signal intensities for the assay targets may be reduced affecting the assay outcomes. A 3ml volume of culture media is required to measure background expression of all targets – this control sample is counted as one of the assay samples.

3) Samples should be supplied in bijoux tubes that are clearly labelled; a simple numbering system of tubes (1-20) is preferred. Results will be labelled with these numbers, however if you would prefer to have results labelled with more detailed sample names these can be provided by e-mail.

4) Once the samples have arrived at HTPU and a sample submission form has been received, your study will be scheduled. We would aim to schedule your run and provide results within 4 weeks, or by a mutually agreed date.

5) On completion of your study we will provide you with the following files:

    - An excel spreadsheet with non-specific (secondary antibody only) levels subtracted and data presented as a simple heat map to highlight changes.
    - Powerpoint presentation with all the slide images.
    - A guide / report to help you interpret your data.
    - The original array images are saved as .tiff files and are available on request.
    - Mapix text files containing all the raw data output are also available on request.

Sample Preparation:

1) Cell culture samples

It is important that steps are taken at the experiment set-up stage to ensure that differences across all the experimental samples are minimized:

- Count cells when setting up the assay plates to ensure consistent plating densities.
- Where possible re-count cells or measure cell protein from lysed cells after sample collection (this may be used for data normalization).
- Ensure all plates have identical media volumes in order to reduce variability.

If serum addition to culture media is necessary, an aliquot of media with the same batch of serum will be required to measure basal levels of all the assay targets.

Collected samples should be stored at -80^{o}C until delivered to HTPU lab for assay.

Example cell culture experimental set up:

Cells plated in 10cm dishes at 1.5-2 x 10^{6} cells per dish. After plating leave the cells for 24hrs to settle then change the media adding 4mls of fresh complete medium. Leave media to condition for 24-48 hours, (this really depends on how actively your cells secrete chemokines). When collecting the media, spin it down at 1000rpm for 5 min and re-tube to remove any debris. Wash the plates of cells with PBS and lyse them in RIPA (or alternative) lysis buffer. Perform a protein estimation which can be used for normalization of the cytokine array data.

2) Blood samples:

a. Serum preparation:

- Collect whole blood in a covered test tube or suitable commercially available tubes (for example a red topped BD vacutainer).
- After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 15–30 minutes. Remove the clot by centrifuging at 1,000–2,000 x g for 10 minutes in a refrigerated centrifuge.
- Immediately transfer the supernatant (serum) to a clean polypropylene tube using a Pasteur pipette. Maintain the samples at 2–8°C while handling.
- Store samples of serum at –20°C or lower in appropriate sized aliquots. It is important to avoid freeze-thaw cycles because this is detrimental to many serum components.

b. Plasma preparation

- Collect whole blood into suitable commercially available anticoagulant-treated tubes; for example EDTA-treated lavender topped BD vacutainer, or citrate-treated light blue topped BD vacutainer.
  N.B. Heparinized tubes (for example green topped BD vacutainer) are indicated for some applications; however, heparin can often be contaminated with endotoxin, which can stimulate white blood cells to release cytokines.
- Cells are removed from plasma by centrifugation for 10 minutes at 1,000–2,000 x g using a refrigerated centrifuge. (Centrifugation for 15 minutes at 2,000 x g may be required for complete depletion of platelets from the plasma sample).
- Immediately transfer the supernatant (plasma) to a clean polypropylene tube using a Pasteur pipette. Maintain the samples at 2–8°C while handling.
- Store samples of serum at –20°C or lower in appropriate sized aliquots. It is important to avoid freeze-thaw cycles.