Nitrocellulose RPPA: Host and Tumour Profiling unit, Cancer Research UK - Edinburgh Centre, IGMM.

If you are considering using the service:

1) The following standard layout options are available:
   - 12 samples printed with 3 technical replicates per sample.
   - 36 samples printed with 1 technical repeat per sample (we only advise this option where 3 biological replicates are provided per assay condition).
   - 36 samples printed with 3 technical replicates per sample.
   - 108 samples printed with 1 technical repeat per sample (we only advise this option where 3 biological replicates are provided per assay condition).

2) Standard antibody numbers: 60 – 120 (for more than 120 antibodies please discuss further with HTPU)

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

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

Running Nitrocellulose RPPA 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) Preparation and quantification of protein samples are performed by the client as detailed on the next page. If samples are not quantified and normalised, an additional charge per sample will be incurred.

3) Ideally, a total of 100µl per sample should be shipped on dry ice to HTPU at 2mg/ml. We can proceed with lower volume (minimum 50ul) and concentration (minimum 1mg/ml) by performing repeated sample deposition rounds but this increases print time and reduces image quality. If sample yields are lower than this we cannot guarantee array image quality.

4) Samples should be supplied in 1.5ml tubes that are clearly labelled; a simple numbering system of tubes (1-36) 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.

5) 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.

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

   - An excel spreadsheet with extracted data from each slide (8 arrays). As well as raw data we will provide Fast-green (total protein stain) normalised data in a graphed format.
   - 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.

Recommended Lysis Buffer Preparation:

Note: Other cell lysis buffers (including RIPA) have been successfully used for RPPA studies.

1) Prepare the following 2x buffer solution:

Reagent	Volume (ml)
Triton X-100	5
0.5M HEPES pH7.4	50
0.5M EGTA pH7.5-8	1
1M Sodium Chloride	75
1M Magnesium Chloride	0.75

Make to 250ml with distilled water. Filter sterilise through a 0.22µM PVDF filter unit and store at 4°C.

2) Prepare the following inhibitor stocks:
   a. **100mM Sodium Orthovanadate (Na_3VO_4):** Add 0.92g to 40ml water. Adjust to pH10 using HCl. The solution will now be yellow. Boil the solution until it becomes clear. Allow it to cool. Check the pH and adjust again to 10, re-boil until colourless. This step may need to be repeated. Aliquot and store at -20°C.
   b. **100mM Tetrasodium Pyrophosphate (TSPP):** Add 2.65g to 100ml water, aliquot and store at -20°C.
   c. **1M Sodium Fluoride (NaF):** Add 4.2g per 100ml water, aliquot and store at -20°C.

3) Prepare 1x lysis buffer fresh prior to use:

Reagent	Volume (ml)
2x buffer solution	5
100mM Na3VO4	0.1
100mM TSPP	1
1M NaF	1
Complete mini EDTA-free protease inhibitor	1 tablet
Phos-stop phosphatase inhibitor tablet	1 tablet
Glycerol	1
Distilled Water	1.9

Once prepared keep on ice.

4) Brief lysis procedure for cell lines (SOP available on request):
   - Wash cells twice with ice cold PBS whilst kept on ice, and remove **all** excess PBS.
   - Add lysis buffer to the cells (suggested volumes are 75-150ul for each well of 6-well plate and 250-400µl for a 10cm dish).
   - Scrape cells into 1.5ml tube and incubate on ice for 20 mins (with occasional vortexing).
   - Centrifuge at 14,000 rpm (maximum speed) for 10 minutes at 4°C.
   - Carefully collect supernatant. Discard the pellet.
   - Store protein at -20/-80°C.

5) Protein determination (SOP available on request)
   - Recommended assay: Coomassie Plus Protein Assay (ThermoFisher Scientific #10741945)
   - Adjust protein concentration to 2mg/ml with lysis buffer. Protein concentrations less than 2mg/ml may be useable but this must be discussed with the RPPA personnel prior to use.