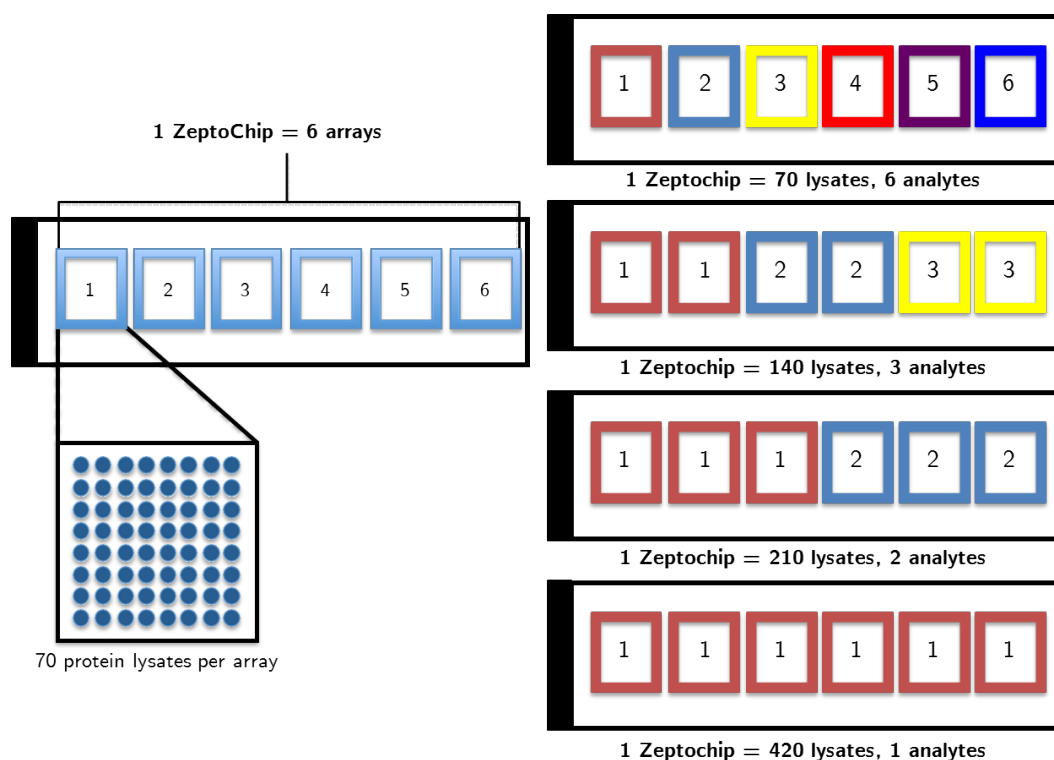


RPPA Platform Pricing Guide

Overview

The platform employs the Zeptosens system for RPPA screens. Protein lysates are spotted onto proprietary ZeptoChips using the GeSim Nanoplotter 2.0. Each chip is separated into 6 arrays which represents an antibody (analyte) and contain up to 66 protein samples. The RPPA platform adds in an additional 4 lysates of known protein expression as controls. We can tailor RPPA runs to suit any number of samples and offer full flexibility in your choice of antibodies to be screened. The figure below illustrates the permutations possible, with option 1 taking up to 66 samples per array the most common application.



Lysis Buffer

Samples submitted for RPPA must be lysed in lysis buffer provided by the VCFG (it is specific for the chip). There are two lysis buffers available: CLB1 (most applications) and CLB96 (for lysing in at least 96 well format), the platform will advise as to which buffer is more suited for your project.

- \$ Lysis Buffer (CLB1 or CLB95): \$5 per 0.5 ml aliquot
e.g. for 66 samples in a 6 well dish format, ~5.25 mL will be needed. Cost: \$55.
See RPPA User guide for lysis protocols

Quantification of Protein Concentration

To spot onto a ZeptoChip for RPPA, the samples must be diluted to a final concentration of 0.2mg/mL in the Zeptosens spotting buffer (provided by the platform). To achieve the correct ratio between lysate and spotting buffer, the platform requests that samples should be a minimum concentration of 1mg/mL in at least 30 µL. The quantification of samples for screening is included in the cost of the screen. Samples quantified for pilot screens or assay development will incur a small charge. Samples that fail minimum requirements can be concentrated in-house using the Amicon concentration columns.

Enabling discovery and driving translational medicine with high throughput technologies



- \$ Protein quantification for assay development: \$5 per sample.
- \$ Samples concentration using Amicon concentration columns: \$8.75 per sample.

RPPA Screen Costs

We offer two screening options. 1) The complete run dedicated to an individual researcher who has sufficient samples or urgency to fill a chip. E.g you have up to 66 samples and choose you're antibodies. You can submit more than 66 samples, we will run arrays in multiples, but defined by the experimental design to ensure like samples and replicates are kept together; 2). A shared run, common for pilot screens, where we match you with other projects that are small scale, you all select your preferred antibodies, you pay to screen those but you get data from all the antibodies screened. You will only receive your data, all other information will be redacted. Note, if no other pilot screens are running, and you wish to proceed then you will incur the cost of a full screen. The platform will determine the sample break point to proceed.

Each RPPA run must include appropriate controls. This consists of secondary antibody alone (one array for each secondary antibody i.e. rabbit, mouse) as well as a housekeeping control against Prohibitin. The combination of samples and antibodies is fully customisable for each project provided that following parameters are met:

The first chip to be screened will have at least 2 arrays dedicated to controls (3 if you are screening with mouse secondaries, only limited number of mouse antibodies in the collection), and thus only 4 antibodies can be selected. Thereafter, antibodies should be selected in multiples of 6.

Example 1 – 1 chip

→ 66 samples

→ Requested Abs:

1. Akt rabbit Ab
2. p53 rabbit Ab
3. FAK rabbit Ab
4. Src rabbit Ab
5. Prohibitin
6. Rabbit 2° alone

Example 2 – 1 chip

→ 66 samples

→ Requested Abs:

1. Akt rabbit Ab
2. p53 rabbit Ab
3. Rap1 *mouse* Ab
4. Prohibitin
5. *Mouse* 2° alone
6. Rabbit 2° alone

Example 3 – 2 chip

→ 66 samples

→ Requested Abs:

1. Akt rabbit Ab
2. p53 rabbit Ab
3. FAK rabbit Ab
4. Src rabbit Ab
5. Prohibitin
6. Rabbit 2° alone
7. PTEN rabbit Ab
8. P27 rabbit Ab
9. RhoA rabbit Ab
10. Rac1 rabbit Ab
11. Brca1 rabbit Ab
12. E-cad rabbit Ab

The RPPA platform operates a tiered cost model based on our current funding arrangements and foundation support partners.

- Tier 1 = Peter Mac, Bio21, Anatomy and Neuroscience, Murdoch Institute
- Tier 2 = All other UoM departments
- Tier 3 = All other research Institutes

Dedicated single researcher RPPA Screen Cost

N.B. The table below is indicative of the total number of antibodies in the platform collection.

Nº of Chips	Arrays Available	Tier 1	Tier 2	Tier 3
1	4	\$ 312.00	\$ 378.00	\$ 409.00
2	10	\$ 533.00	\$ 644.00	\$ 698.00
3	16	\$ 759.00	\$ 918.00	\$ 994.00
4	22	\$ 979.00	\$ 1,185.00	\$ 1,283.00
5	28	\$ 1,206.00	\$ 1,459.00	\$ 1,579.00
6	34	\$ 1,426.00	\$ 1,725.00	\$ 1,868.00
7	40	\$ 1,647.00	\$ 1,993.00	\$ 2,157.00
8	46	\$ 1,873.00	\$ 2,266.00	\$ 2,453.00
9	52	\$ 2,094.00	\$ 2,533.00	\$ 2,742.00
10	58	\$ 2,320.00	\$ 2,807.00	\$ 3,038.00
11	64	\$ 2,540.00	\$ 3,074.00	\$ 3,328.00
12	70	\$ 2,766.00	\$ 3,347.00	\$ 3,624.00
13	76	\$ 2,987.00	\$ 3,614.00	\$ 3,913.00
14	82	\$ 3,207.00	\$ 3,881.00	\$ 4,202.00
15	88	\$ 3,434.00	\$ 4,155.00	\$ 4,498.00
16	94	\$ 3,654.00	\$ 4,421.00	\$ 4,787.00
17	100	\$ 3,880.00	\$ 4,695.00	\$ 5,083.00
18	106	\$ 4,101.00	\$ 4,962.00	\$ 5,372.00
19	112	\$ 4,322.00	\$ 5,229.00	\$ 5,661.00
20	118	\$ 4,548.00	\$ 5,502.00	\$ 5,957.00
21	124	\$ 4,768.00	\$ 5,770.00	\$ 6,246.00
22	130	\$ 4,994.00	\$ 6,043.00	\$ 6,542.00
23	136	\$ 5,215.00	\$ 6,310.00	\$ 6,832.00
24	142	\$ 5,441.00	\$ 6,583.00	\$ 7,128.00
25	148	\$ 5,661.00	\$ 6,850.00	\$ 7,416.00
26	154	\$ 5,882.00	\$ 7,117.00	\$ 7,706.00
27	160	\$ 6,108.00	\$ 7,391.00	\$ 8,002.00
28	166	\$ 6,329.00	\$ 7,658.00	\$ 8,291.00
29	172	\$ 6,555.00	\$ 7,931.00	\$ 8,587.00
30	178	\$ 6,776.00	\$ 8,198.00	\$ 8,876.00

Shared RPPA Screen Cost

The cost of Shared RPPA screen will be divided amongst the projects selected, prorated on the number of samples submitted and the number of antibodies requested. As such, the cost will be dependent on the projects available. We will endeavour to give you a more accurate representation of cost prior to the run. *NOTE this is a representative example of how we share, the number of samples adds up to 70 based on our former protocols, this is now changed to 66 but doesn't alter the concept we are trying to illustrate.*

Scenario 1 of Shared Screen:

Project A: submitted 35 samples and requested 5 rabbit 1° antibodies

Project B: submitted 15 samples and requested 11 rabbit 1° antibodies

Project C: submitted 20 samples and requested 5 rabbit & 1 mouse 1° antibodies

Total number of chip run: 4

Project:	A	B	C
No. of Samples:	35	15	20
Antibodies Requested:	5	11	6
Run Control:	1	1	2
Tier 1 Price:	\$344	\$363	\$273
Tier 2 Price:	\$417	\$440	\$331
Tier 3 Price:	\$451	\$476	\$385

Scenario 2 of Shared Screen:

Project A: submitted 12 samples and requested 5 rabbit 1° antibodies

Project B: submitted 4 samples and requested 13 rabbit & 3 mouse 1° antibodies

Project C: submitted 15 samples and requested 11 rabbit 1° antibodies

Project D: submitted 15 samples and requested 13 rabbit & 3 mouse 1° antibodies

Project E: submitted 24 samples and requested 17 rabbit 1° antibodies

Total number of chip run: 7

Project:	A	B	C	D	E
No. of Samples:	12	4	15	15	24
Antibodies Requested:	5	16	11	16	17
Run Control:	1	2	1	2	1
Tier 1 Price:	\$195	\$269	\$312	\$383	\$490
Tier 2 Price:	\$236	\$326	\$378	\$464	\$593
Tier 3 Price:	\$256	\$353	\$409	\$502	\$642



Analysis

Bioinformatics support is included in the cost of the screen as follows:

- A report on quality control markers for the screen.
- A normalized spreadsheet of relative fluorescence values.
- A graph of relative fluorescence values for all sample for each antibody analysed.
- A detailed guide to options for analysis.

\$ Any further analysis will be performed on a contract basis with the VCFG Bioinformatician and is charged at \$75 per hour.

Western Blots

The RPPA platform also provides western blotting services as a standalone service or in addition to any RPPA project. The RPPA lysis buffer is compatible with western blotting, we run 4-12% SDS-polyacrylamide gels (Thermofisher) followed by transfer onto PVDF membrane. We can use all antibodies from the platform or BYO. Target specificity is visualised via infrared secondary antibodies using a LI-COR Odyssey CLx Infrared imaging system. We also have multiplex apparatus (MPX system, LI-COR) that uses 200µL of primary antibody and can probe up to 20 different antibodies at once.

The platform has a large bank of cell line lysates and can provide antibody validation via WB. The submitted antibodies will be run on up 4 different lysates (Determined in conjunction with the researcher.)

- \$ WB Option 1: 1 Lysate, 20 primary antibodies: \$427 per gel
- \$ WB Option 2: 11 Lysate, 11 primary antibodies: \$367 per gel
- \$ WB Option 3: 11 Lysate, 2 primary antibodies: \$318 per gel
- \$ WB Option 4: 11 Lysate, 3 primary antibodies: \$323 per gel
- \$ Validation of Researcher submitted antibody for RPPA: \$50 per antibody