Conventional RayBiotech L507 Human Antibody Array Protocol

DAY 1

1.1 Add 10ul serum and 90ul 1X PBS to a 1.5 mL microcentrifuge tube and centrifuge MAX speed for 5 min. Put in dialysis tube and dialysis in 1L of 1X PBS with a stirring stick in the cold room at 4 degree. After 3 hours change PBS, dialysis O/N

DAY 2

2.1 Transfer serum from dialysis tube to a 1.5 mL tube and spin down MAX 5 min

2.2 For each reaction in a new 1.5 mL tube add 22 ul Labeling reagent Item B (first add 100ul 1X PBS to solubilize) 35 ul Dialyzed serum 155 ul Labeling buffer Item K

Vortex

2.3 Put on rotator at RT for 30 min

2.4 Add 3 ul Stop Solution Item D into reaction tube

2.5 Vortex and centrifuge MAX 5 min

2.6 Dialysis in PBS O/N 4 degree

2.7 Take out a new array from -20. Equilibrate in the seal at RT for 15 min. Take it out of the seal and put in hood for 1-2 hours. Add 400ul Blocking Buffer each subarray and seal and let block O/N at 4 degree

DAY 3

3.1 Spin the dialyzed sample 5 min MAX speed

3.2 To each subarray, add 360 ul Blocking Buffer + 40 ul sample (10x dilution)

3.3 Seal, O/N at 4 degree

DAY 4

4.1 Wash with buffer I 800ul each subarray 3 times for 5 min Put entire array in a container, cover with Buffer I and wash 2 times for 10 min with shaking Cover with Buffer II and wash 2 times 5 min shaking

4.2 Prepare Cy3 Conjugated Streptavidin Spin down Cy3-Conjugated Streptavidin Add 1000ul of Blocking buffer and resuspend gently to make the Cy3-Conjugated

Streptavidin stock Take 400ul of the Cy3 stock solution, add to 1600 ul Blocking Buffer 4.3 Add 400ul of diluted Cy3-conjugated Streptavidin to each subarray. Cover with seal and then aluminum foil. Incubate O/N at 4 degree

DAY 5

5.1 Discard Cy3 solution and dissemble slide and put in tube. Wrap tube in foil. Wash 3 times Buffer I 10 min each, then 2 times Buffer II 5 min each, then 1 time DI water 5 min 5.2 Dry and scan