SurVeyix Biotinylated Antibody Plates

For research use only. Not for diagnostic or therapeutic procedures

www.InCepix.com P: 651 560 2015 info@incepix.com

Antibody Catalogue in a Plate: SurVeyix Antibody Plates provide access to large catalogues of antibodies in a simple to use format. Unlike antibody arrays which require special equipment and readers, SurVeyix plates provide antibody in a preserved state within the wells of a 96 well plate. Antibody is available for staining of components directly in the well, or can be used outside of the well after reconstitution in buffer. Below is a map of the wells in SurVeyix Plates (SU-001). Antigen specificity of antibody in each well is listed. Further clone and performance data is available at Ancell.com.

Reconstitution Volume: Individual wells of the SurVeyix plate contain preserved biotinylated antibody. Biotinylated antibodies are deposited at 0.2 ug/well. The reconstitution volume allows for some control over your working concentrations. For cell surface staining, 50 ul of cell suspension added to well results in a staining concentration of 4 ug/ml which is at or above saturation concentrations for cell surface staining. Adding 100 ul of buffer yields a 2 ug/ml working concentration.

Liquid Handling: An 8 channel pipette allows for quick reconstitution of the individual wells. For timed incubations a multi pipette is suggested as it allows cells to be rapidly added to each well. Care should be taken when pipetting in and out of the wells to avoid cross contamination, as each well contains different antibody. Clean pipette tips must also be used to after wells are reconstituted to prevent contamination.

Cell Surface Staining Protocol: Your own protocol for cell surface staining can be used or modified to take advantage of the SurVeyix plates. If you have access to a multi well plate centrifuge and FACs plate handler, the entire assay can be run in the SurVeyix plates themselves. The SurVeyix plates have flat bottomed wells and it may be advantageous to move cells with antibody into u or v bottom plates and can be easily done with a multi-channel pipette.

The following protocol can be used for cell surface antigen detection using SurVeyix Antibody Plates. Cells are stained in the wells directly and then transferred to individual FACs tubes for further washing and reading.

Step One: Antibody Binding. Process cell sample as normal, ie. washing and resuspension in FACs buffer. Resuspend cells to 4 x 10^6 cells/ml in FACs wash buffer. Dispense 50 ul of cell suspension into each well of the SurVeyix plate. Use of 8 channel multi pipettor is recommended. This will create a 4 ug/ml concentration of biotinylated antibody in each well containing cells. To assure complete reconstitution of preserved antibody, place the reconstituted plate on a plate shaker and shake for 5 min. Following shaking, the plate is placed at 4 C or on ice to complete a 60 minute incubation.

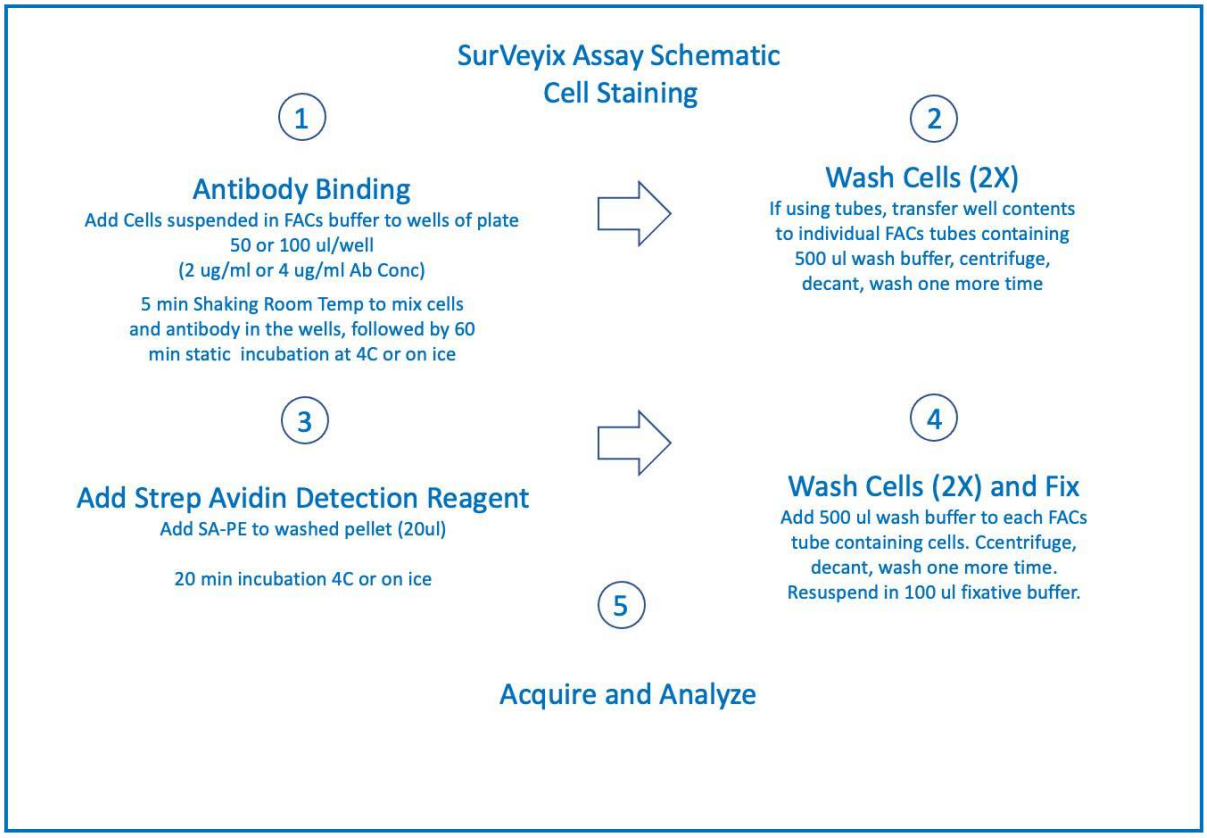
Step Two: Wash 2X. At this point in assay, well contents are transferred to individual FACs compatible plastic tubes that can be read manually on a FACs machine. Alternatively, if a plate handler is available for your FACs machine, the assay can be ran in multi well plates. If a u or v bottom well is desired for centrifugation and washing, transfer well contents into your desired plate format.

For FACs tubes, add 500 ul of FACs wash buffer to each tube. Transfer well contents to FACs tubes using clean pipette tip. Set pipettor to greater than 50 ul, to assure maximum volume recovery from each well. Spin FACs tubes to pellet cells and decant wash buffer. Add 500 ul of wash buffer again to tube to resuspend cell pellet and centrifuge to pellet cells.

Step Three: Detection Antibody Binding. At this step the biotinylated antibodies are bound to the cell surface proteins and excess antibody has been washed away. To detect bound antibody a secondary anti biotin specific reagent is needed. In this assay a streptavidin conjugated phycoerythrin (SA-PE) is used to detect bound biotinylated antibody. The PE signal is acquired by FACs and analyzed. Prepare sufficient volume of SA-PE to add 20 ul per assay tube. SA-PE should be diluted according to the source. Normal working dilution of your SA-PE solution for FACs is satisfactory.

Add 20 ul of SA-PE to each assay tube and vortex to mix. Incubate on ice in dark for 20 minutes.

Step Four: Wash 2X. Add 500 ul of wash buffer to each tube and centrifuge to pellet cells. Decant wash buffer and wash one more time by adding 500 ul wash buffer to cell pellet, vortex, centrifuge, decant. Cell pellet can now be resuspended in buffer or fixative. To fix, cell pellet is resuspended in 100 ul formaldehyde containing fixative buffer. Vortex to mix. Store fixed cells in the dark at 4C until analysis.



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WELL	1	2	3	4	5	6	7	8	9	10	11	12
A	CD1a	CD7	CD14	CD25	CD33	CD41	CD49d	CD59	CD69	CD83	CD105	CD134
B	CD1b	CD8	CD16	CD26	bcl-2	MHCII	CD50	CD62e	CD70	CD84	CD106	CD137
C	CD1c	CD9	CD18	CD27	CD35	CD43	CD51	CD62L	CD71	CD86	CD117	CD137l
D	CD2	CD10	CD19	CD28	MHCI	CD44	CD53	CD62P	CD74	TCRCb1	DR3	CD147
E	CD3	CD11a	CD20	CD29	CD37	CD45	CD54	CD63	CD79a	CD94	CD122	CD152
F	CD4	CD11b	CD21	CD30	CD38	CD45RO	CD55	CD64	CD79b	CD95	CD127	CD154
G	CD5	CD11c	CD22	CD31	CD39	CD46	CD56	CD66b	CD80	CD98	Perforin	CD155
H	CD6	CD13	CD23	CD32	CD40	CD48	CD58	CD66e	CD81	CD104	CD133	p53

Plate One

WELL	1	2	3
A	CD161	CD273	LTbR
B	CD166	CD274	TIGIT
C	CD244	CD279	mIgM
D	CD252	CD326	
E	CD268	357GITTR	
F	CD270	CD66B	
G	CD271	GARP	
H	CD272	TCRVb3	

Plate Two