EVix Multi-Tope Kit

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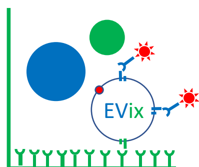


The EVix Multi-Tope ELISA kit captures EVs with 115 unique antibodies in individual wells. The captured EVs are detected with a mix of anti CD9, CD63 and CD81 biotinylated antibodies. Each kit contains 115 different antibody specific capture wells in duplicate, including CD9, CD63 and CD81 in multiple wells for a total of 256 individual assay wells,. Duplicate capture wells are run with and without EVs providing a negative control for each capture antibody.

Use to phenotype EV preparations by antigen content. Multi-Tope kits are used to characterize EV preparations from different sources or production runs. A detailed finger print of antigen presence and levels are obtained. Data from the Multi-Tope assay can be further examined using alternative detection antibodies in the Single-Tope kit.

Kit Contents: If contents are unopened and stored at 4C and -70C the performance of the kit is stable for 6 months.

A: Multi Tope Capture Plates: Three (MT1, MT2 and MT3) 96 well plates (Store 4C) containing 256 capture antibody coated assay wells.
B: 10X Sample and Wash Buffer Concentrate: 1 bottle (40 ml) to make 400 ml. (Store 4C)
C: Biotinylated Primary Detection Antibody: 1 vial of 1.5 ml to make 30 ml. (Store -70C)
D: Horse Radish Peroxidase Conjugated Streptavidin(SAHRP): 1 vial of 1.5 ml to make 30 ml. (Store -70C)
E: Chromogenic Solutions : 1 bottle (30 ml) of a stabilized H2O2 , 1 vial (1.5 ml) of stabilized TMB chromogen (Store 4C) **Caution!**
F: Stop Solution: 1 bottle (30 ml) **Acid Caution!** (Store RT)

Incubation Time and Temperature: An 18 hour EV capture incubation at 4 C is recommended. A 1 hour detection incubation with detection antibodies at room temp is sufficient. Shaking is necessary during all incubations to assure maximum interaction between the EV and the capture antibody. If you alter these times, control them to reproduce your results.

Calibration: Prior to assay, the EV sample should be calibrated using the High Sensitivity Tetraspan Tracking (HSTT) kit to determine presence of EVs in sample. Samples should be sufficiently concentrated to generate HSTT signals near or above 3.0 Absorbance Units (AU) 450 nm. A sample volume of 13.0 ml is needed to run the complete 256 well Multi Tope assay.

Assay Protocol: An 18 hour incubation to capture EVs is followed by a 1 hour detection antibody incubation followed by a 20 minute SAHRP incubation. Wells are washed once after detection antibody incubation and three times after SAHRP incubation. The assay should be run in total to reduce inter-plate variation, each plate has control tetraspan signals which can be used to track inter-plate variation.

1. Capture EVs (18 hours): Dispense 100 ul of 1X wash buffer into the odd numbered columns of the three Multi Tope capture plates for negative controls. Plate 1 and 2 have 96 antibody coated capture wells each for use. Plate 3 has 64 antibody coated capture wells for use and the last 4 columns (9,10,11,12) are blank wells. Dispense 100 ul of EV sample into wells in the even numbered columns of the Multi Tope plates. Use of a multichannel pipettor is advised if available. Incubate plates for at least 18 hours at 4 C with continuous shaking. Use 1X wash buffer to dilute sample and for the negative controls. Refer to map below to fill plates. After 18 hour capture, aspirate the well volume. The well is ready for the next step, the addition of detection antibody.

2. Detection antibody (1 hour): Add 100 ul of diluted biotinylated detection antibody solution to each well in the assay. 1 hour incubation at room temperature with shaking has been shown to be optimal. Detection antibody is diluted 21 fold for use. Add 1.5 ml of detection antibody solution into 30 ml of wash buffer. This is sufficient to add 100 ul of solution to each well. After 1 hour incubation (shaken) , aspirate the well volumes. Add 300 ul of wash buffer to each well and aspirate. The empty well is ready for the next step, addition of 100 ul Streptavidin Horse Radish Peroxidase (SAHRP).

3. SAHRP (20 minutes): Add 100 ul of diluted SAHRP solution to each empty well. SA HRP is diluted 21 fold before use. To make 30 ml of SA HRP add 1.5 ml (vial contents) of concentrate to 30 ml of 1X Wash Buffer. Plates should be shaken at room temp for **20 min**.

4. Wash 3X: After SAHRP incubation, wells receive 3 x 300 ul washes. Well contents are aspirated and 300 ul of 1X Wash Buffer is added. This is done 3 times and final volume is aspirated prior to addition of chromogenic substrate.

5. Chromogen Addition (10 minute): The chromogenic solution is prepared by adding 1.5 ml of part A to 30 ml of part B. **CAUTION: These components are considered hazardous.** Add 100 ul of chromogenic solution to each washed well. The enzymatic reaction is carried out for 10 min and should be timed, to provide equivalent results from assay to assay. Blue color will develop in positive wells.

6. Stop Solution: Add 100 ul of stop solution to all wells after the above 10 minute incubation. Wells will change from blue to yellow upon addition of the stop solution. **CAUTION. Stop solution is 1N Sulfuric Acid and should be handled accordingly.**

7. Read Absorbance: Read the optical absorbance of each well at 450 nm wave length within 10 minutes after addition of the stop reagent.

Data Analysis: The map below shows the capture antibody used for each well in the Multi Tope plates. The wells are set up with negative controls (no EV) in the odd numbered columns next to the sample wells in the even numbered columns. The background signal for each capture antibody is subtracted from the sample signal to yield ABS value above background.

	1 , 2	3 , 4	5,6	7,8	9,10	11, 12
A	CD9	CD63	CD81	Null	CD9	CD63
B	CD1a	CD6	CD13	CD22	CD30	CD37
C	CD1b	CD7	CD14	CD23	CD31	CD38
D	CD1c	CD8	CD16	CD25	CD32	CD39
E	CD2	CD10	CD18	CD26	CD33	CD40
F	CD3	CD11a	CD19	CD27	bcl-2	CD41
G	CD4	CD11b	CD20	CD28	CD35	MHCII
H	CD5	CD11c	CD21	CD29	MHCI	CD43

MT1 Plate 1

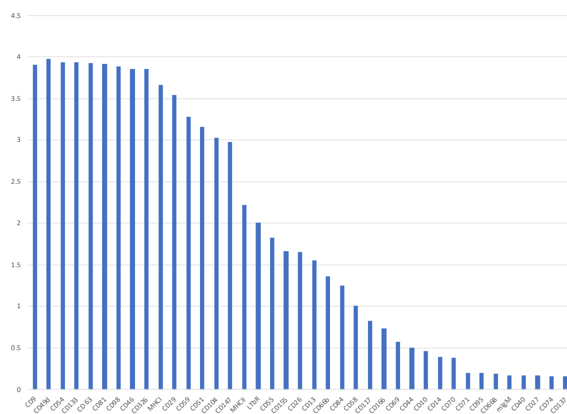
	1 , 2	3, 4	5,6	7,8	9,10	11, 12
A	CD81	Null	CD9	CD63	CD81	Null
B	CD44	CD51	CD62e	CD70	CD84	CD105
C	CD45	CD53	CD62L	CD71	CD86	CD106
D	CD45RO	CD54	CD62P	CD74	TCRCb1	CD117
E	CD46	CD55	CD64	CD79a	CD94	DR3
F	CD48	CD56	CD66b	CD79b	CD95	CD122
G	CD49d	CD58	CD66e	CD80	CD98	CD127
H	CD50	CD59	CD69	CD83	CD104	Perforin

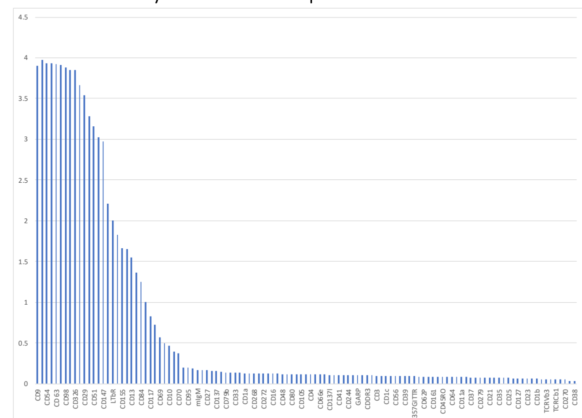
MT2 Plate 2

	1 , 2	3, 4	5,6	7,8
A	CD9	CD63	CD81	Null
B	CD133	CD155	CD270	357GITTR
C	CD134	p53	CD271	CD66B
D	CD137	CD161	CD272	GARP
E	CD137l	CD166	CD273	TCRVb3
F	CD147	CD244	CD274	LTbR
G	CD152	CD252	CD279	TIGIT
H	CD154	CD268	CD326	mIgM

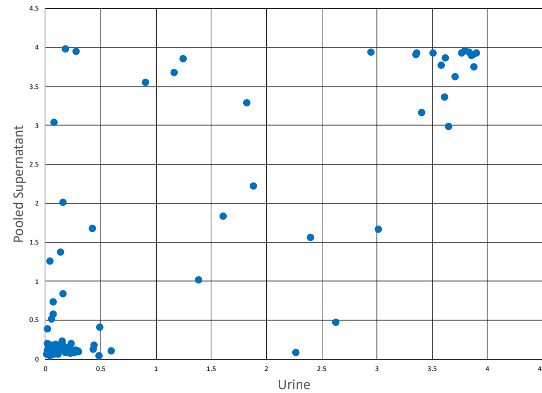
MT3 Plate 3

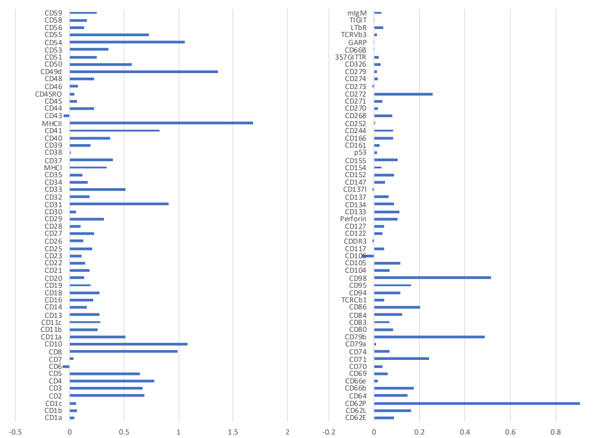
Calibration of sample via EVix HSTT kit prior to assay assures sufficient EVs are present for evaluation. As seen in the values below, CD9, CD63 and CD81 all give near maximum signals. All signals can be ranked and those above a set threshold can be examined and compared to other populations from different samples or sources. Shown below is a sample from pooled culture supernatants with values ranked. The left panel displays only values greater than 0.150. The right panel shows all values with background subtracted. Comparing different EV populations can be done by examining the presence of the top antigens. Different samples and sources yield different profiles.





X Y scatter plots can be created using the Multi-Tope assay. Comparison of two different populations is done by finding signals that may be in one population but not the other. These signals will fall along the axis distinct from signals that are present in both populations. As shown in the panel on the right, there are EVs in urine which are positive for a particular antigen but they are not present in the pooled supernatant sample. Other antigens are represented at similar levels in both populations. Additionally, while some antigens may be present in both populations, they may be expressed at different levels.





Multi Tope antigen patterns differ significantly depending upon EV source. As shown to the left, 10X concentrated and dia-filtered (300 MWCO) supernatant from a human mixed lymphocyte culture on day 7 yield signals not seen in Urine or serum samples. As would be expected, there are more immune associated antigens detected. The Multi Tope assay creates a detailed fingerprint of tetraspan associated antigens. This complex phenotype analysis can be used to confirm similarities in various EV preparations. This is valuable in manufacturing control.

Having identified antigens in EV populations using the Multi Tope kit, relevant antigens can now be further characterized. Use the EVix Selecta-Tope kit to characterize the antigen profile of your different positive populations. While all positive populations express tetraspans, the next question is what antigens are expressed on the specific identified populations. The Selecta-Tope kit allows the user to capture with the Multi Tope plates but to screen with the biotinylated detection antibody of choice.